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CLINICAL DIAGNOSIS

A TEXT-BOOK
of
CLINICAL MICROSCOPY AND CLINICAL CHEMISTRY
FOR MEDICAL STUDENTS, LABORATORY
WORKERS, AND PRACTITIONERS
OF MEDICINE

BY

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156 ILLUSTRATIONS

FIFTH EDITION ENTIRELY REWRITTEN AND RESET

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no 1

To

WILLIAM OSLER, M.D.

IN GRATEFUL RECOGNITION
OF THE MANY KINDNESSES
RECEIVED BY A PUPIL AND
ASSISTANT, THIS BOOK IS
AFFECTIONATELY DEDICATED
BY THE AUTHOR

PREFACE TO THE FIFTH EDITION

Ten years have passed since the last edition of this book appeared and seven years since its last copy disappeared from the market, periods of time which in personal history may seem short, since crowded by so many momentous events, but in the history of a text-book is only too long, since this Clinical Diagnosis now must present itself as entirely new and win whatever success it may, assisted little by the popularity it formerly enjoyed. The author desires to express his appreciation to the many friends who gave the previous editions such cordial reception and invites them now to examine critically this.

As regards its contents this certainly is a new work. So much new material has appeared during the past ten years that a complete rewriting of the entire volume has been necessary and several new sections have been added, among them, those on serology, bacteriology, chemistry of the blood and of the spinal fluid.

In presenting this edition to the inspection of the reader, the author takes the liberty to outline the general plan he has followed in its preparation and to state the ideals which have influenced him. As in the case of the previous editions this book is not merely a manual for laboratory workers but is intended especially for medical students and for those practitioners of Internal Medicine whose conscience urges them to do their own laboratory work or at least personally to supervise and to interpret all the laboratory examinations made for each of their cases. The author feels that the present separation of laboratory and ward is an evil which cannot be too strongly condemned. No matter how successful a man may be he has no right to entrust his laboratory work entirely to others. Laboratory assistants who can do the mechanical work and the coöperation of specialists in the medical science are necessary to the clinician but the conscientious man must regard the laboratory examinations as part of the general personal examination and must hold himself personally responsible for them all. The one who takes the history of the patient and makes the physical examination is the only one who can interpret correctly a laboratory finding. Exactly identical reports may have quite different meanings in two different cases. He alone who knows the patient can interpret and evaluate a specimen under a microscope or in a test-tube and he often sees there that for the record of which no dotted line is provided on a laboratory blank but which will suggest further questions for the history and further physical examinations which may be of great value. The rather widespread and blind confidence which the past generation has placed in impersonal laboratory reports has brought Internal Medicine into a certain degree of disrepute.

Medicine is an art, it cannot be a science. While it is not a science, it has found all the sciences very useful in its development as an art. The medical student should understand that the sum total of all laboratory activities is not and never can be internal medicine and that he as artist must try to see in each specimen something of the patient himself and interpret it in terms, not of chemistry, physics or biology, but of the suffering person.

No attempt has been made to give a wide review of literature or to describe methods as yet untried. Those are recorded which the author and his associates personally have found valuable. This book is not the product of one man but of the teachers, associates and assistants of the Johns Hopkins and Indiana University Medical Schools with which it has been the privilege of the author to be associated. It is obviously impossible for him justly to acknowledge his indebtedness to the many who consciously or unconsciously have assisted him. He will, however, mention gratefully by name, among those who have assisted directly in the preparation of this edition, Dr. Virgil H. Moon, Prof. B. Bernard Turner, Dr. John R. Thrasher and Dr. Harry K. Langdon.

Indianapolis, July 1, 1921

PREFACE TO THE FOURTH EDITION

THE changes made in the preparation of this new edition are numerous and important. Our attempt has been to incorporate such important new contributions to the laboratory diagnosis of diseases as belong in a book of this nature, and to omit methods which have been replaced by better ones. These omissions and the grouping of illustrations formerly in the text in plates have made it possible to preserve for the most part the paging of the third edition. The most extensive additions and alterations are those in the chapters on tuberculosis and lues. No effort has been spared to make this book accurate and practical. We would acknowledge with gratitude the helpful assistance of our many friends, among whom may be mentioned Dr. Clyde G. Guthrie of Baltimore, Dr. Roger Morris of St. Louis, Dr. John R. Thrasher of Indianapolis, Dr. Louis W. Ladd of Cleveland and Dr. H. S. Houghton of Wuhu, China.

CHARLES P. EMERSON.

INDIANAPOLIS, 1913.

PREFACE TO THE FIRST EDITION

There have, during the past few years, appeared so many and such excellent text-books on clinical diagnosis, the clinical examination of the blood, the urine, or the gastric contents, that to add to this number one which covered the same ground in the same way as they, would seem a thankless undertaking, as well as an unpardonable misuse of energy. It is because the present work tries to cover this same ground in a different way, and one which will, we believe, commend itself to the medical profession, that we venture to offer it for inspection; we refer to the consideration of clinical laboratory work from the clinical rather than from the laboratory point of view.

This book is based on the author's experience as physician in charge of the clinical laboratory, and instructor in medicine, of the Johns Hopkins Hospital and University. He has also had at his disposal all the clinical records of the ward cases for the seventeen years of this hospital's activity.

Our course in clinical microscopy and chemistry extends over the eight months of the student's third year; two afternoons of three hours, and one of one hour, each week; but much of the work is done out of class hours, as inspection of pages 447 and 485 will show. The subjects studied are the clinical examination of the blood, urine, sputum, stomach contents, feces, and various fluids, as ascitic, pleural, cerebrospinal, cyst contents, etc. In addition to this the student follows cases assigned him in the outpatient department. To those fitted for such work simple problems of research are given. The course is a laboratory one; specimens are provided each of the students. It is needless to say that with the eighty microscopes focussed on eighty specimens of a patient's blood, sputum, etc., the most of the interesting cells or other features will be found. The best were drawn by an artist always within call. The questions discussed in the following pages are for the most part those asked by the students during the class-work. The object of this course is not so much to impart knowledge as to raise the efficiency of the student. It is not a course in chemistry and microscopy, but in these applied to the study of a patient; not in physiology, but in pathology. With the methods of chemical and biological work, with the normal findings, they are already familiar. Chemistry, inorganic and organic, qualitative and quantitative, is required for admission to the school; the normal blood they have studied in the anatomical laboratory; normal urine and gastric contents, in the laboratory of physiological chemistry. We take this knowledge for granted as a foundation for the study of pathological bloods, urines, etc., paying particular attention to the clinical significance of these findings. At the same time the students are required to practise the best methods in every-day use, not only until they understand them, but until they can accurately use them. It is the practical use of a determination or examination which is emphasized. If approximate methods will do, they are used; if accurate methods are necessary, accurate work must be done, whatever the cost in time. To use an approximate method well is far better than to employ a more exact, laborious one poorly; to do approximate work is not always easy and requires practice; to be able to do accurate work well is also required of our students. Practice, experience, an exact knowledge, first of the possibilities in a method, second, and just as important, his own accuracy in the use of that method—these it is the duty of the clinical laboratory to give a student. Above all, he should train his common sense so that, using his eyes, nose, ears, and tongue, he can get results for which another man would apply elaborate methods.

The author has been careful not to include new untried methods, for of these but a small number will last, and a text-book should contain nothing as yet not well tested by friends and foes. It is the introduction of " new methods " which renders some books even dangerous to the man who buys but one.

We do not claim that with this book alone the student can study clinical microscopy. No subject in medicine is broader or requires more reference books, for some of the hardest chemical problems will at times confront him, and to interpret the various artefacts and accidental findings of the microscope would require a vast experience in microscopy, and a knowledge of zoölogy, botany, and mineralogy as broad as is the realm of science. For who knows what infusoria, what diatom, desmid, or other protophyte, the ovum of what parasite, the wing of what insect, the leg of what fly, the tissue of what plant, the fiber of what meat, the seeds of what berry or fruit, may be found in sputum, stomach contents, urine or feces, from the food, tap-water, or the contaminations from dirty vessels, or from the dust of the air ? To be wise in the points of differential chemical and microscopical diagnosis is splendid; but to recognize artefacts and extraneous matter, the stumbling-blocks in diagnosis, that is the true test of the clinical laboratory worker, and this ability is gained by wide experience alone.

The function of the clinical laboratory worker is to aid the ward worker. The findings of the former are seldom conclusive, and must be interpreted in the light of the ward findings; especially is this true now that functional diagnosis is the goal. The writer can only give to the reader who has aspirations to be a clinical chemist and microscopist the advice in substance which one of Germany's greatest clinical chemists gave him when the latter regretfully left the little Swiss laboratory which had been such a pleasant home: the clinical chemist must be first a good clinician and second a chemist; he should remember that even from the laboratory point of view his stethoscope is of more importance than his microscope, his percussion finger than his whole outfit of chemical apparatus.

In conclusion, we wish to express our indebtedness to Doctor Osler for his encouragement and aid during the progress of this work, and for his hearty coöperation in placing at our disposal the records of the medical wards; and to the assistants and students of this clinic, for whose aid I am very grateful, and who are too many to mention by name except Dr. Thomas R. Boggs, whose suggestions and criticisms have been so valuable.

I take this opportunity to thank the artists who have done much beautiful work for me—Messrs. F. S. Lockwood, Hermann Becker, Max Brödel, and Mrs. Ruth Huntington Brödel, whose excellent half-tone and pen-and-ink drawings must be recognized by the lack of her signature.

Charles P. Emerson.

Johns Hopkins Hospital, 1906.

INTRODUCTION

THE clinical laboratory has two special functions in the medical school,—in it the student learns the application of physical and chemical methods in the study of disease, and in it researches are conducted on the innumerable problems concerning etiology, diagnosis, and treatment. Forming an essential part of the hospital-half of a school, it should be close to the wards and so arranged as to have ample facilities for the students and for the house physicians and others doing special work. It should be in charge of a man resident in the hospital, familiar with the routine of the clinic, and in close daily touch with his chief and with the assistants. The expenses should be shared equally by the hospital and the medical school. Into the details of organization I will not enter, but the director of such a laboratory should, if possible, have assistants thoroughly trained in bacteriology, physiological methods, and physiological chemistry.

In 1896, through the kindness of two ladies, a special clinical laboratory was built for the students of the Johns Hopkins Medical School, which was enlarged two years ago when the new clinical building was erected. On each of the two floors about fifty students are accommodated and there are rooms adjacent for special workers and for the assistants. Dr. Jesse Lazear was at first in charge, and under Dr. Thayer's direction the well-known researches of Macallum and Opie and of Lazear himself on malaria were carried on. In 1900, after Dr. Lazear went to Cuba, we were fortunate enough to have Dr. Charles P. Emerson take charge of the laboratory, and to him the medical school is deeply indebted for the organization of clinical laboratory courses of the most thorough and scientific character.

In medical education the all-important problem is to give a man the knowledge he can use. In our modern system much of the training is rendered ineffective, as it has not been sufficiently prolonged to become part of a man's intellectual or bodily mechanism. A brief course of six weeks on any practical subject is almost useless and in some may be positively dangerous. When possible, an orderly sequence should be followed, so that the work of each year shall supplement that of the preceding. In the seven-year course laid down by the Johns Hopkins University a thorough laboratory training in biology, physics, and chemistry is given before the professional work begins, so that a man enters the medical school proper with a practical knowledge of scientific methods and of the use of instruments of precision. In his first year of the medical curriculum the courses in histology and physiology and in the second year those in physiology, bacteriology, physiological chemistry, and pathological histology give him an insight into the structure and functions of the body, and he becomes thoroughly familiar with the use of all instruments of precision. In the third and fourth years in the hospital side of his education, for which the previous ones have been a preparation, he must have opportunities to carry on his practical work, and these the clinical laboratory affords. A student who has been interested in the mysteries and mechanism of cardiac rhythm in the physiological course should be able to take the pulse and heart tracings of the first case of mitral disease that he meets in the out-patient department, and the means should be afforded him to pass without a jar from the normal to the abnormal,—without, indeed, appreciating that there is any difference in the method of approaching the problems involved. So too a student should be able at once to attack his first case of diabetes as a problem in carbohydrate metabolism, fully prepared by previous study to approach it on the clinical side.

If the curriculum were not so full, a student could gradually work out for himself, as the patients came under observation, every detail in the application of scientific methods to clinical study, but it is found more convenient to group them together and present in orderly sequence the subjects for study. Concurrently with the systematic instruction in the out-patient department which forms a large part of the work of the third year, a course on microscopical and chemical methods is given, and each man has his own place in the laboratory at which he may work throughout the year. This book is the outcome of the work by Dr. Emerson and his students in this course during the past five years. Not only does it represent the results of a very large number of careful observations made in the laboratory, but an analysis of many important groups of cases in the wards, so that it illustrates the experience of the medical clinic of this hospital so far as it relates to microscopical and chemical methods of diagnosis. The work will be found a comprehensive and trustworthy guide in all the details of laboratory work.

But the aim of a training such as this book implies is to send out into practice men able to give patients the benefit of modern scientific methods in the diagnosis and treatment of disease—men who *use* the microscope, who examine sputum, and who *use* the stethoscope, and who can do the routine urine and blood work with confidence. The men to study a book of this kind are the young practitioners who are keeping up the practical knowledge obtained in the medical school, and who appreciate a small laboratory as the most valuable stock-in-trade. As a practitioner becomes more and more engaged, he can hand over to an assistant the laboratory side of the work, but it is surprising how much can be done even by the busiest of men if the *will* is there and if the methods have once been thoroughly mastered.

WILLIAM OSLER.

January 30, 1906.

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CLINICAL DIAGNOSIS

CHAPTER I

THE SPUTUM

Introduction.—The examination of the sputum is fast becoming a lost art. The discovery of a few specific microörganisms and the hope of finding many more, the American desire for a "sure" test and his enthusiastic study of new subjects, the "latest things" in medicine, all have led him gradually to abandon a great and valuable fund of knowledge. Today a sputum examination seldom means more than the search for Bacillus tuberculosis and that by a State laboratory worker. That the average clinician now does not even glance at fresh sputum is proven by the presence on the market of red paper sputum cups only; red, so that the patient may not notice that he is expectorating blood; red of such tint that the doctor cannot see the many delicate shades and characteristics of sputum which would have taught our fathers in medicine much of interest and value concerning the patient. There certainly are many other diseases of the lungs than tuberculosis and even in that one disease our duty involves not only an accurate diagnosis but an accurate prognosis as well and that we may be able to do this we must follow our case in its changes from day to day. For this reason a wise interpretation of the daily sputum changes is more important than a report from a State laboratory or a röntgenological department. Our fathers, who never saw a germ, could diagnose and follow some pulmonary cases better than can we. They would not have made some of the mistakes which now humiliate us. The variety of colors, of physical and chemical characteristics and of structures, which the sputum may present or contain, is bewildering. Some of these are very important, more are negligible; which are which, the clinician should know. He should not mistake bacteria in chains for elastic tissue, nor myelin globules for blood-cells. For a would-be Bizzozero to have expensive pictures of ordinary starch granules drawn, confident that a newly-discovered parasite will bear his name, probably means that when a medical student he did not study fresh sputum with enthusiasm.

GENERAL FEATURES

The sputum is, strictly speaking, that substance or mass of substances which is expectorated. The term covers not only the secretions from the respiratory passages but also any constituents added to these from the esophagus, nose and mouth, or, through perforation into these, from any neighboring organ.

The normal person should theoretically have no sputum and yet persons who live in an atmosphere laden with dust may every morning expectorate lumps, often as large as a cherry, which are tough, elastic, so translucent that they resemble boiled sago (due to myelin) and gray in color from coal dust. Microscopically "morning sputum" consists of mucus, in which may be detected viscid streaks arising from the goblet-cells and more watery portions from glands. Embedded in this sputum are rows of cells, both epithelial and pus, loaded with coal pigment and myelin. In addition are non-nucleated cell-like masses (probably degenerated epithelial cells) and pus-cells clumped together in balls. These latter as a rule contain no pigment.

Sputum is raised from the trachea by coughing unless there is so much that it actually flows from the mouth. But there are some patients who, although they should furnish sufficient sputum for examination, persist in swallowing it. Most of these are children, or persons of filthy habits, or partially unconscious patients. The doctor is often rewarded for the time he spends urging such patients to expectorate.

One of Doctor Osler's assistants created amusement by sitting persistently at the bedside of a case with pneumonia and begging her to expectorate. At last he obtained a very little sputum, but it contained tubercle bacilli, and the hospital record for the early diagnosis of the pneumonic type of pulmonary tuberculosis was broken.

Among the ways to get the swallowed sputum are, by washing out the stomach, and, in children, by examining the stools. Findlay recommends that in the case of young children the examiner cover his finger with gauze and with it tickle the child's pharynx to stimulate coughing. As the sputum rises in the throat it can be caught on the gauze.

The patient must be carefully taught to avoid expectorating into the specimen cup saliva, nasal and pharyngeal mucus, food, etc.

Amount.—A record of the amount of sputum expectorated each 24 hours is sometimes of aid in following the progress of a disease. In some rare cases, although the cough is severe, the bronchial secretion is so scanty and so viscid that practically none is expectorated. This occurs in "dry" bronchitis, often in diffuse bronchitis, in incipient tuberculosis, in post-influenzal bronchopneumonia, rarely in acute lobar pneumonia and caseous pneumonia. Large quantities of sputum are expectorated in some cases of chronic bronchitis, of advanced tuberculosis with large cavities, of bronchiectasis, of pulmonary gangrene, while in cases of edema of the lung, of albuminous sputum following thoracentesis, of pulmonary hemorrhage, of perforating pleural exudate and of rupturing lung abscess the serum, blood or pus evacuated through the bronchial tree may literally pour from the mouth and may even drown the patient.

The clinical chemist engaged in metabolism experiments must remember to take an abundant sputum into account since this may contain even 5% of the total nitrogen eliminated.

Consistency.—Generally speaking, the consistency of sputum varies inversely with its total amount and directly with the amount of mucin it contains. Early in an attack of true bronchial asthma, in acute bronchitis and in pertussis the sputum is scanty and may be very tenacious. When, on the contrary, it is abundant and contains little mucin and much water, as in edema of the lungs and in chronic bronchitis which has denuded the bronchial tree of its mucous membrane, the sputum is very watery. A marked exception to the above rule is the sputum of croupous pneumonia, which, though abundant, will not spill from the inverted cup (see page 61).

Reaction.—Fresh sputum is alkaline in reaction, but that which has stood for some time in the cup, or which has stagnated in the body, is usually acid, the result of bacterial fermentation.

Character.—Mucoid sputum is glairy, transparent, tenacious and becomes cloudy on the addition of acetic acid (due to mucin). Such sputum is seen in acute bronchitis, pertussis, and early asthma.

A mucopurulent sputum consists of mucus which contains pus-cells in sufficient numbers to cloud it. There are two forms of mucopurulent sputum. The first consists of clear mucus containing streaks and dots of pus while in the second the mucus and pus are more intimately mixed. If in the latter case the pus is present in small amount it gives the mucus a faint white haze; if it is relatively more abundant, a yellow or yellowish-green tint; if very abundant, the sputum may resemble pure pus.

Purulent sputum is said to differ from pure pus in that the former contains mucus and so is more tenacious. But a purulent sputum may contain practically no mucus at all, as in the condition known as bronchoblennorrhea, present when the bronchial tree has been practically denuded of its mucous membrane. On the other hand the sputum certainly consists of almost pure pus when an empyema of the pleura perforates through the lung, or an abscess of the lung ruptures into a bronchus, or an abscess of a neighboring organ discharges through the lung, trachea, esophagus, or nasal passages.

Serous sputum is watery and colorless and because of its high percentage of albumin foams easily. One meets with it in edema of the lung, in perforating serous pleurisy, and in rare cases following thoracentesis.

Color.—Bloody sputum may consist of almost pure blood or may gain its name from a much less, even a very slight, blood content. Such sputum may be due to trauma of the chest, hemorrhagic infarction of the lung, pulmonary gangrene, acute lobar pneumonia, caseous pneumonia (early), pulmonary tuberculosis, tumors of the lung, intense chronic passive congestion, or to "weeping" aneurism. As a rule the blood is mixed with mucus and air and hence appears frothy. It may escape from the capillaries by diapedesis, in which case it signifies a severe inflammation of the mucosa, or it may pour from a ruptured vessel.

Sputum containing the DERIVATIVES OF HEMOGLOBIN may be of almost any color. Formerly it was taught that variations in the number of blood-cells explained this variety of colors, but Traube proved that unchanged erythrocytes can color the sputum only red. Blood-cells free in the alveoli, bronchi, or lung tissue, however, soon disintegrate and the various oxidation products of their hemoglobin give the sputum that wide range of color—the various shades of red, brown, green, orange, yellow and chocolate seen, for example, in a subcutaneous bruise. Intact blood-cells in greater or less number may be found in such sputum, but the majority are pale and swollen. The best known example of sputum colored by modified free hemoglobin is the rusty sputum of pneumonia, the rusty color of which is due to an unknown derivative of hemoglobin. Another good example is the somewhat similar sputum which follows a small hemorrhage into the lung tissue or into a pulmonary cavity, while still another is met with in chronic passive pulmonary congestion, especially that due to mitral valve disease, in which sputum the characteristic light brown streaks and dots are due to masses of alveolar epithelial cells loaded with amorphous granules of modified blood pigment (Hertzfehlerzellen). The sputum of patients in whose lungs are areas of necrotic lung tissue permeated with blood, such as occur in pulmonary gangrene, lung abscess or infarction, may have a uniform, dirty brown color due to masses of hematoidin crystals. Similar crystals are sometimes found in the sputum of cases with chronic passive congestion of the lungs. While the bile-stained sputum of a jaundiced person usually has no significance, yet the bile-stained sputum of a person who is not jaundiced may have value as in case of a liver abscess perforating through the lung. Such sputum may contain bilirubin or biliverdin.

A definitely GREEN SPUTUM always demands an explanation. The pure mucoid sputum of a jaundiced patient with bronchitis may have a fine grass-green color due to biliverdin. Sputum of just the same color is seen in some patients (not jaundiced) during the lysis of an ordinary croupous pneumonia, in cases of pneumonia ending in abscess and in subacute caseous pneumonia. (It is interesting that Traube, Gesam. Beitr., ii, p. 699, 1871, first called attention to green sputum in "pneumonia" and cited only cases of caseous pneumonia.) Grass-green sputum may occur also in chloroma of the lung; while, finally, certain chromogenic bacteria may give the sputum this same color.

The sputum in the various PNEUMOKONIOSES deserves particular mention. The most common of these is *anthracosis* or induration of the lungs due to inhaled coal-dust. While the best examples of this are seen in coal miners, yet lesser grades are very common among city residents. The sputum in this condition usually is dirty gray, sometimes quite black.

Many granules, escaping expectoration, penetrate the bronchial mucosa, while others are swallowed and later make their way to the interlobular pulmonary lymph-channels, which they render beautifully visible. It is stated that granules deposited in the interlobular tissue are never expectorated unless freed by a destructive pulmonary process, yet some coal miners without any symptom of tuberculosis have continued to expectorate a black sputum for years after changing their occupation (Osler). *Siderosis* is due to the long continued inhaling of metallic dusts and occurs among workers in iron, bronze, brass, etc. The best examples, however, are furnished by mirror polishers, whose sputum is red with ferric oxide. Those who inhale much mineral dust suffer from *chalicosis*, called also "stone-cutters' phthisis," "grinders' rot," etc. These patients have very contracted chests and for years may have frequently recurring non-tuberculous pulmonary hemorrhages. Practically all, sooner or later, become tuberculous. They are susceptible to various other pulmonary infections, as gangrene, which frequently leads to pneumothorax. Their sputum contains much of the mineral dust they inhale.

Those who work with dry dyes, as methylene blue, have deeply colored sputum; bakers expectorate doughy masses; cotton-mill operatives expectorate masses of cotton; while particles of tobacco and of colored foods, drinks, medicines, are often found in the sputum of patients and may deceive the unwary.

Finally, CHROMOGENIC BACTERIA, such as B. virescens, B. pyocyaneus and many others, which produce "sputum cup ward infections" may materially change the appearance of sputum.

Air is present in the sputum in bubbles of varying sizes depending on the diameter of the bronchi furnishing the sputum, and on the efforts required to expel it. Sputum from lung cavities and from the larger bronchi may contain no air and hence will sink in water. This "sputum fundum petens" was formerly considered conclusive proof of a lung cavity but is met with also in acute tracheitis.

Layer Formation.—The tendency of a sputum to separate into layers (layer formation) may be of assistance in diagnosis. In certain conditions, especially bronchorrhea, bronchiectasis, putrid bronchitis, and gangrene of the lung, the sputum is abundant, and, if collected in a tall jar, will separate into three layers: an upper, of frothy mucus; a lower, of morphological elements, *i.e.*, pus, tissue shreds, detritus, etc.; and a middle of the pus-serum, usually a cloudy, watery fluid. Often from the under surface of the upper mucous layer a sufficient number of streamers to constitute a fourth layer, consisting of the same material as the sediment, hang down in the pus-serum.

Odor.—Fresh sputum is usually almost odorless. Sputum allowed to stand and that which has stagnated in the body soon gain a very positive odor. In tuberculosis and bronchiectasis the odor is heavy, sweet and penetrating; that of a perforating empyema is said to resemble old cheese; that of putrid bronchitis and of many cases of bronchiectasis is fetid; and that of gangrene is generally the worst of all. In pulmonary tuberculosis the odor of a patient's breath may be fouler than that of his sputum after it is cold. Some have claimed to have diagnosed on this evidence small tuberculous cavities before the physical signs of ulceration had appeared.

MACROSCOPIC CONSTITUENTS

Small MASSES OF PUS-CELLS are common in the sputum, their size indicating roughly the size of the bronchi from which they come.

Fragments of necrotic tissue may be found in the sputum of cases of abscess or gangrene of the lung. These are sometimes quite large, even 2 cm. long; those in tuberculous sputum are, as a rule, small, almost at the limit of vision. The fragments from a pulmonary abscess are yellow in color since permeated by pus-cells, those from other conditions may be dark from changed blood or black from coal pigment. Their nature is determined by the presence of elastic tissue. The discovery of even the smallest fragment of elastic tissue is important, for it proves the presence of an ulcerating lesion of the lung. To find these fragments the sputum should be squeezed out between two glass plates and studied with a hand lens. They are most numerous in the nummular masses from tuberculous cavities (see page 58). Fragments of necrotic cartilage from tuberculous ulcers of the larynx, trachea, or bronchi are sometimes found in the sputum. In a case of typhoid fever both arytenoid cartilages were expectorated. The demonstration of tumor fragments in the sputum may lead to the correct diagnosis of a doubtful condition.

Dittrich's plugs are short cylindrical casts of bronchi, some scarcely visible, others as large as a bean. The size of the majority ranges from that of a millet seed to that of a mustard seed. The small plugs are opaque, yellowish-white and the larger ones dirty gray in color. If crushed between the fingers they emit a horrible odor. Microscopically, they consist for the most part of zoöglea of bacteria, fatty acid crystals, fat droplets and cell detritus. They may contain also pigment granules, fragmented red corpuscles and hematoidin crystals. Flagellated protozoa and a leptothrix not yet thoroughly studied, but which takes a fine blue stain with iodine solution, have been found in them. The fatty acid crystals may be long and curved, or short, fine needles. The plugs contain but few intact cells. In some, perhaps fresher than the others, a few leucocytes can be recognized. These plugs may be found in the sputum of any putrid bronchial disease, especially in putrid bronchitis and bronchiectasis, in which latter disease they are especially large. How these are formed we do not know (Hoffmann), but the presumption is that they come from the smaller bronchi which open into a diseased or dilated larger bronchus. Somewhat similar plugs, shaped like a beechnut, come from the crypts of the tonsils.

Curschmann's spirals (Fig. 1) are perhaps the most beautiful structures met with in the sputum. They are found in practically every case of true bronchial asthma at some time during its course. They have been reported also in the sputum of rare cases of acute bronchitis, acute lobar pneumonia, chronic pulmonary tuberculosis and in certain rare, interesting cases in which were combined the features of bronchial asthma and fibrinous bronchitis. In the sputum of one such case were found small fibrinous bronchial casts, the tips of whose branches were directly continuous with the central fibers of typical spirals. Curschmann considered the spirals the result of a bronchiolitis exudativa.

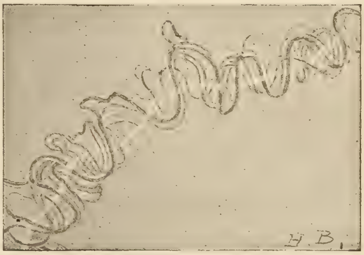

FIG. 1.—Curschmann's spiral, from the sputum of a case of asthma. × 200

Two forms of spiral may be described. The first is a spirally twisted strand of mucus enclosing many leucocytes, especially the coarsely granular cells, and Charcot-Leyden crystals. The more beautiful form consists of a tight skein of mucus, the "mantle," wound around a "central fiber." These spirals are from 1 to 2 cm. or more long and 1 mm. broad. They may be branched. In the mantle are clumps of coarsely granular leucocytes, pigmented epithelial cells, ciliated cells and Charcot-Leyden crystals. The central fiber, made up probably of transformed mucus, is a very refractive, spirally twisted strand, uniform in diameter, with a smooth contour or saw-edge. Some central fibers are small, from 0.5 to 1μ in diameter; some medium-sized, 3μ; while others are thick, from 3 to 18μ in diameter. Ruge, who studied sputum hardened and cut into sections, found all these fibers solid, none with the lumen which others had described. Not all central fibers are as conspicuous as this. Some spirals have but a trace of a central fiber, others none. Some fibers end in a thread, others give off many lateral threads which radiate to the mantle. These finer fibers are often branches of larger ones. Some of the larger central fibers are lamellated, while others seem to be bundles of parallel threads, spirally twisted. These central fibers are not mere optical phenomena. They may partially project from the mantle, or lie in the sputum quite separate from it, and then are often twisted spirally. When alone they have the same significance as complete spirals. In the sputum of some cases one finds perfect spirals; in others, central fibers, some free and some with very imperfect mantles (see Fig. 1), while in still others free central fibers only (Fig. 2).

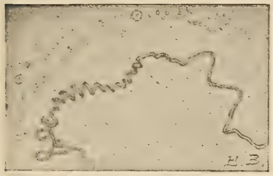

FIG. 2.—Free central fiber of a Curschmann's spiral, from the sputum of a case of asthma. × 200.

The origin of the spirals is not understood. The central fibers certainly are not casts of the smaller bronchi, since their diameter is usually but one-tenth as great.

Schmidt considered that the conditions necessary for the formation of spirals were well preserved bronchial epithelium and a tough mucous secretion. He thought that the central thread represented the most twisted and therefore the most compact part of the spiral. Hoffmann claimed that the smaller bronchi are themselves spirals, which straighten out each time the lung expands, and that therefore tough mucus forced through them would assume a spiral form. Others claim that the cilia of the bronchi create spiral currents along the bronchi; others, that a straight band of mucus moving along a bronchus is whipped into a spiral by the tangential motion of the cilia of another bronchus at the point where these two unite. Gerlach stated that the three conditions necessary for their production were a small amount of very viscid sputum, very forcible respiratory movements and clear bronchi, three conditions which are best complied with in asthma. He claimed that the mantle and the central fiber both are formed at the same place in the bronchus but the central fiber later and is merely an optical expression for that part of the mucous mass which has been twisted the most. We deny this emphatically. The central fibers are separate structures which may be found free of the spiral; they are themselves twisted bands of fibers with markedly fewer revolutions per unit of length than the fibers of the mantle surrounding them.

We have studied beautiful spirals about 2.5 cm. long and 1 mm. broad, with central threads so refractive that they could be definitely seen with the naked eye. These central fibers or cores consisted of bundles of twisted fibers but the mantles surrounding them were much more tightly twisted. Some cores were bundles of fibers with very few turns indeed. It was evident on cross section that the mantles were composed of spirally wound sheets of mucus each enclosing various cells: squamous epithelial cells, very many alveolar cells, some containing coal pigment and others modified hemoglobin, leucocytes especially eosinophiles, and cylindrical cells, some with the cilia indistinctly seen and some of them goblet-cells. It was of interest that these cells were not mingled, but each variety occurred in groups or lines, large numbers in each group. The Charcot-Leyden crystals occur singly or in clumps. Some were quite large. One projected from a disintegrating eosinophile cell. In some fields full of leucocytes we searched in vain for one which was not coarsely granular. In one field was a strip of mucosa of cylindrical epithelium. Some spirals had no core while others consisted merely of a very large refractile central fiber. One spiral was particularly interesting. It consisted of two strands of mucus, the inner thick, the outer thin, each rich in cells nearly all of which were coarsely granular leucocytes. These two strands, separate at one end, were twisted, the one within the other, into a spiral. For some little distance in the spiral each strand could be traced separately but the coil became tighter and tighter until they could no longer be distinguished. If the spirals are allowed to dry slightly the central thread becomes much more distinct.

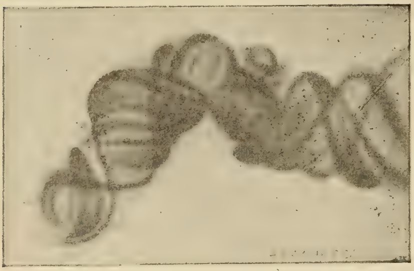

FIG. 3.—A spiral thread of mucus from the sputum. × 5.

The sputum of another typical case of asthma contained spirals, immense numbers of eosinophile cells and a large number of epithelial cells, some of which were alveolar cells containing the various forms of pigment, while others were cylindrical cells, both ciliated and goblet in type.

In another case were many central fibers, some without mantles and others with a few fibers of a mantle wound around them. The fibers forming these imperfect mantles were remarkably thread-like and of quite uniform diameter. Many of the coarse strands of mucus found in the sputum of bronchitis are spirally twisted (see Fig. 3).

Fibrinous Structures.—Under the title "fibrinous structures" we include all formations popularly supposed to consist of fibrin, although some contain none. In diphtheria one sometimes finds masses of whitish membrane in the sputum, which, if they come from the throat, larynx, or trachea, have no distinctive shape, but if from the bronchi they may be arborescent casts. In the sputum of pneumonia casts of the bronchial tree are often found, most of them small, but some very large (see page 63, and Fig. 25). These are brownish or reddish in color and contain blood and many leucocytes. Acute fibrinous bronchitis with similar casts in the sputum may accompany various fevers, such as typhoid fever, erysipelas, measles, smallpox, scarlet fever and acute articular rheumatism. It may also accompany exophthalmic goiter, pulmonary tuberculosis and mitral disease. Similar casts have been found in the albuminous expectoration after thoracentesis and after the inhalation of irritating vapors and gases. But the most interesting cases are those of chronic idiopathic fibrinous bronchitis, which condition has been well reviewed by Bettmann[1] (see page 73). In the sputum of these cases, together with well formed arborescent casts and sometimes alone, one finds also unformed masses of the same material, evidently also from the bronchi.

Whether any of the material in many of these structures is really fibrin or not is very doubtful. The tests for fibrin usually used are: the physical properties of the mass (its color, toughness, etc.), its tendency to swell and clear on the addition of acetic acid (which precipitates mucin) and the rapid effervescence on the addition of hydrogen peroxide. Hirschkowitz found, however, that one cast expectorated by a case of tuberculosis did consist of pure fibrin.

Casts made up of the mycelium of fungi may be expectorated. In Osler's case a small cast consisted of a mass of aspergillus mycelial threads. Similar casts were expectorated for years by the case reported by Devilliers and Renon.

Lung Stones.—Almost any mass in the sputum which is hard enough to wound the bronchial mucosa when it is expelled is called a "stone," whether cartilaginous, caseous, or calcareous in nature. The term should, however, be limited to masses of tissue or of inspissated exudate impregnated with lime salts which, dissected free by pyogenic infection, become foreign bodies in the air passages.

ENCHONDROMATA and OSTEOMATA of the bronchi and lungs may be demonstrated *in situ* at autopsy, but among Poulalion's cases[2] we could find mention of none in which they were found in the sputum. At autopsy we find also pulmonary infarcts, areas of bronchopneumonia, miliary abscesses, the pseudotubercles of actinomycosis, cladothrix, or moulds, cyst walls, cyst contents and tumors which have become calcified, yet we know of no case in which a patient has expectorated calcified fragments of such tissues. Among the calcified masses which have been expectorated are fragments of calcified bronchial cartilage (Fraenkel) and of a calcified blood-clot (Hoffmann). But the vast majority of lung stones found in the sputum are masses of calcified tuberculous tissue. These have been classified in two groups, bronchioliths and pneumoliths.

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[1] American Jour. Med. Sci., Feby., 1902.
[2] Thesis, Paris, 1891.

BRONCHIOLITHS are formed by the deposit of lime salts in the stagnated contents of a bronchus or bronchiectatic cavity. Some of these concretions have as nucleus a foreign body, for example, a cherry-stone or a grain of wheat. A few are arborescent; most of them are irregular and jagged and vary in size from that of a millet seed to that of a bean. They may be chalky or stony hard. Some "resembling coral, finely ramified and very hard" have been described. In one case the stone weighed 0.47 gm. and had 10 or 12 branches. In Atlee's case[3] the stone was ¾ of an inch long and ¼ of an inch wide at the larger end.

PNEUMOLITHS may be fragments of calcified caseous areas of lung or masses of the calcified contents of a closed tuberculous cavity, or, and these are most numerous, fragments of calcified tuberculous bronchial lymph-glands. These masses are treated as foreign bodies and, with the aid of secondary pyogenic infections, ulcerate into a bronchus. In some of the first the structure of the lung tissue itself, even a few cell nuclei and tubercle bacilli, may be seen in sections of the decalcified mass. There are two distinct varieties of pneumoliths: the *cretaceous*, which are chalky in consistency, and the *calcareous*, which as a rule are small, hard and have a rough, rounded surface. Their size varies much. Some are as small as a millet seed while others the size of a pigeon's egg cannot be passed by the bronchus until they have disintegrated into fragments.

Chemically, lung stones, whether pneumoliths or bronchioliths, contain for the most part calcium and magnesium combined with carbonic, phosphoric, and sulphuric acids, with traces of ferric oxide and other metals. In some several minerals may be demonstrated, while others seem composed of but one calcium salt. It is seldom that one can say that a stone is a bronchiolith unless it is branched, or a pneumolith unless tissue structure can be demonstrated. Patients usually expectorate but one stone, seldom 2 or 3, but some have expectorated very many, even 200 and in one case 500. Poulalion suspects that all these were fragments of one large single concretion, while Hoffmann and others assumed in such cases a constitutional abnormality, an increased elimination of lime salts through the lungs, and named this condition "pseudophthisis calculosa." Repeated hemorrhages, "hemoptysis calculosa," often accompany the "bronchial colic" when the stone is expelled and are due to trauma of the mucosa. As a rule these hemorrhages are scanty, but some are severe. The presence of these stones may lead also to pulmonary abscess, pulmonary gangrene, or pneumothorax.

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[3] Stern, Deutsch. med. Wchschr., 1904, No. 39; Carlyon, Brit. Med. Jour., 1890, ii, p. 1474.

The largest concretion I have seen was expectorated by a medical confrère after a period of eight months of ill health which at first suggested tuberculosis, then "asthma" and then for several weeks, pulmonary abscesses. This measured ½ inch in length and weighed 14 grains. His improvement following this illness was surprising.

One of my patients, a young woman 38 years old, suspected of tuberculosis, expectorated 22 such stones, irregular in shape, the largest weighing 2⅝ grains and all together weighing 13⅝ grains. The illness which precedes the expectoration of these stones is doubtless due to the secondary infection which sets the stones free.

Among foreign bodies sometimes found in the sputum may be mentioned teeth, cherry-stones, and coins (see also page 78).

Fragments of the wall of echinococcus cysts or the daughter cysts themselves may be expectorated.

MICROSCOPIC CONSTITUENTS

The **microscopical examination** of fresh sputum though easy and often valuable is much neglected. A little sputum is spread upon a plate the base of which is half black and half white. The interesting particles are picked up on a hatpin and squeezed between a cover-glass and slide. It is important that the observer recognize at a glance the extraneous structures, among which may be mentioned particles of food, particularly crumbs of bread, pieces of orange pulp or other fruits, drops of milk, bits of jams and preserves, fruit skins, tobacco, particles of meat containing elastic tissue which may lead to error in diagnosis and fragments of vegetable leaves. It is important to recognize also various threads, particularly fibers of vegetable tissue, linen, cotton, wool and silk (see Fig. 4).

Cells.—The PUS-CELLS of the sputum are usually polymorphonuclear finely granular leucocytes. As seen in the fresh sputum they are spherical and from 7 to 10μ in diameter. The majority are degenerated and contain fat globules, pigment granules, or glycogen granules. In asthma and in a form of bronchitis which has long been known as "eosinophilic bronchitis" the coarsely granular cells may greatly predominate. While their presence in great numbers suggests bronchial asthma it is of little value in diagnosis and on the other hand the absence of these cells certainly is not in favor of tuberculosis.[4]

To demonstrate these cells the sputum is spread on a slide, dried in the air and fixed over the flame. While still warm the slide is immersed for 5 minutes or longer in a 0.5% alcoholic solution of eosin, washed in water and then counterstained for two minutes with a concentrated aqueous solution of methylene blue.

The various EPITHELIAL CELLS found in the sputum demand careful study. *Pavement cells* may have come from the mouth, the pharynx, and the respiratory tract as low as the vocal cords. It is a valuable lesson for the student to scrape from the surface of the tongue a little epithelium and study the masses of these cells from the papillæ among which are imbedded large zoöglea of bacteria. *Ciliated cylindrical epithelium,* together with many goblet-cells, may come from the nose, trachea, or the bronchial tree. Cells with their cilia still intact are seldom seen in the sputum except in cases of asthma, of acute ulcerative processes and of very acute bronchitis. They soon lose their cilia. In very acute cases of asthma the sputum may contain clumps and even rather large sheets of cylindrical epithelium with the cells still ciliated.

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[4] Hilderbrandt, Münch. med. Wchr., 1904, vol. 3

The *alveolar epithelial cells* present an interesting study. They are found in considerable numbers in nearly every sputum examined whether normal or pathological. They assume a large variety of forms and are often difficult to recognize. Until recently their origin was in doubt (see Hoffmann, Nothnagel's System, "Die Krankheiten der Bronchien"). In general these cells are from 4 to 5 times the size of a leucocyte, are oval, their protoplasm coarsely granular, and with one or several large, oval, vesicular nuclei. They are numerous in the sputum of cases of bronchitis which would indicate that in this condition the alveoli are involved as well as the bronchi. They occur most abundantly in the sputum of patients with inflammatory processes involving the alveoli, especially tuberculosis. In some cases of tuberculosis, however, these cells may fill the alveoli and yet none be found in the sputum. They are said to be ameboid on the warm stage; they certainly are active phagocytes. Some of these cells contain *coal pigment* (Fig. 5, *a*), that is, black granules, all of which are supposed, perhaps without sufficient proof, to be particles of carbon. The origin and composition of these granules were long disputed, but finally in one cell a single black granule was found which was unquestionably a particle of charcoal. It is these cells laden with inhaled dust which gives to the morn-

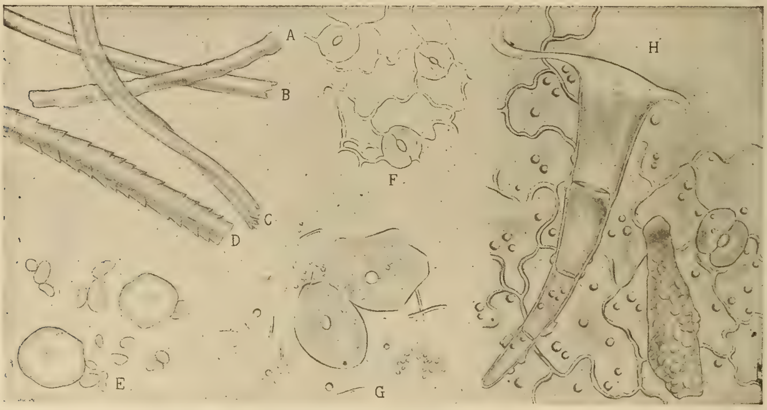

FIG. 4.—Extraneous matter common in the sputum. Threads of, *A*, linen; *B*, silk; *C*, cotton; *D*, wool; *E*, starch granules; *F*, guard cells from a lettuce leaf; *G*, squamous epithelium from tongue, with bacteria attached; *H*, tobacco, showing the surface of the leaf, the large cells stored with oil, and a spine from the surface. × 200.

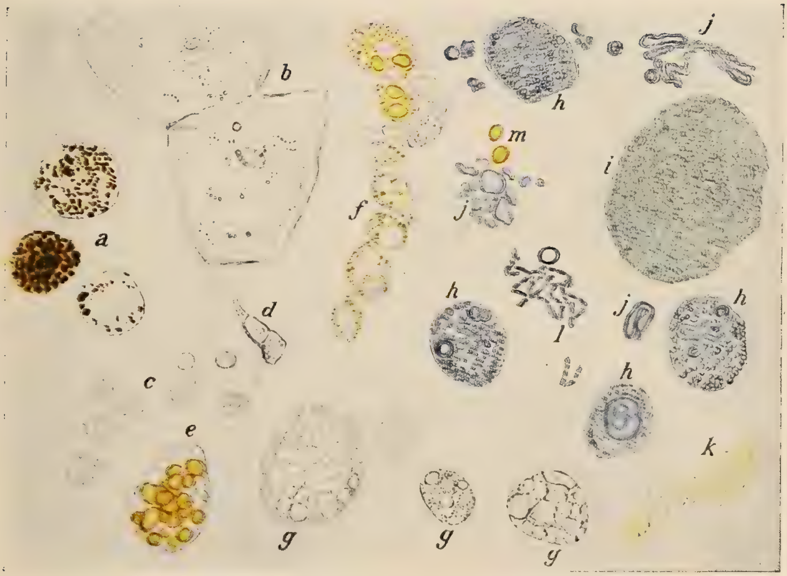

FIG. 5.—Cells in the sputum. *a*, alveolar epithelium cells containing coal-dust; *b*, squamous epithelium cell; *d*, cylindrical epithelial cell; *e* and *f*, Herzfehlerzellen; *g*, cells showing a peculiar degeneration; *h*, those with myelin droplets; *i*, one full of fat droplets; *j*, free myelin; *k*, red blood-cells; *l*, bacteria; *m*, free blood pigment. × 400.

ing sputum its dirty gray color. When very abundant the sputum containing them is smoky or dirty green. Some pulmonary conditions formerly bore the name "phthisis melanotica" because of the abundance of black pigment in the sputum. The *fat globules* (Fig. 5, *i*) which some of these cells contain are spherical, very refractile and glistening. Other alveolar cells contain one or more *myelin globules* which are irregular in shape, often concentrically marked, only slightly refractile, and have a dull greenish or bluish tint. These globules may be very minute in size, others are so large that one of them practically fills the cell (Fig. 5, *h*). Myelin is said by some to be a product of the degeneration of the protoplasm of the cells containing it, but others consider it a normal secretion of the bronchial mucosa, droplets of which these phagocytic alveolar epithelial cells ingest. Cells containing myelin are often numerous in the morning sputum of healthy persons, are very abundant in bronchitis and influenza, while the cases of "desquamatory catarrhal pneumonia" get their name from the sputum which resembles boiled sago because of the masses of alveolar cells filled with myelin droplets. Free myelin is sometimes present in such large amounts that it even exceeds the mucus in quantity, although in general the excretion of these two bodies runs approximately parallel. Free myelin is present in pale, non-refractive drops which vary much in size and more in shape (Fig. 5, *j*); some are concentric spheres, others club-shaped masses. The number and the size of these drops increase as the sputum stands. They resemble in appearance the myelin drops of nerve tissue. Similarly appearing and like named droplets are found in the urine and stools. The use of the name "myelin" in all these cases does not imply a chemical identity but merely a superficial resemblance. Small droplets of certain oils, of fatty acids, and of various neutral fats have the same appearance as these myelin droplets (Liebreich). The myelin of the sputum consists chiefly of protagon, cholesterol and lecithin. It swells a little in water, is not destroyed at 100° C., is stained yellow by iodine but poorly by aniline dyes, is not blackened by osmic acid, is easily soluble in alcohol and slightly soluble in ether and in chloroform. The alveolar cells which contain *derivatives of hemoglobin* are of particular interest. Modified hemoglobin may be present in amorphous granules, in scales of a brownish color, or as hematoidin crystals. "Hertzfehlerzellen" (see Fig. 5, *f*) is the name given to alveolar epithelial cells filled with golden yellow granules of a derivative of hemoglobin, provided these cells are found in large numbers and over a long period of time; only then have they any diagnostic importance. These granules, which vary much in size, are not opaque or deeply colored but rather translucent. Certain cells seem to be diffusely stained by this substance. In chronic passive congestion, especially that due to mitral disease, these cells may be numerous enough to give the sputum a uniform rusty color, but more commonly they are clustered in masses which form dots and streaks of a reddish-brown color in the mucus.

These cells may be found in the sputum in any condition in which red blood-cells escape into the alveoli. They are therefore numerous in chronic pulmonary congestion pneumonia, infarction of the lung and after pulmonary hemorrhage.

The *red blood-cells* (see Fig. 5, *k*) found free in the sputum are often well preserved, many are pressed or drawn out into long threads, but more are represented by masses of amorphous hemoglobin and of further modified pigment. The intact erythrocytes often are crowded into lines and masses in the mucus and are recognizable only by their color.

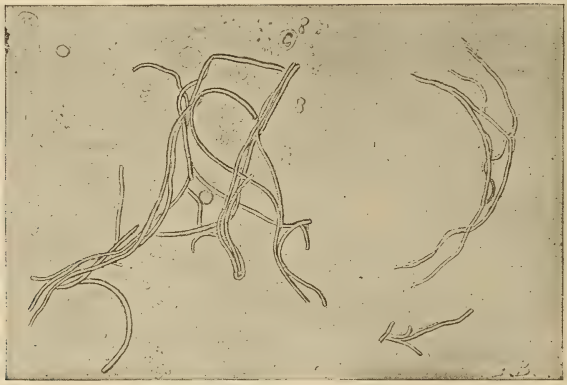

Fig. 6.—Elastic tissue from lung. × 400.

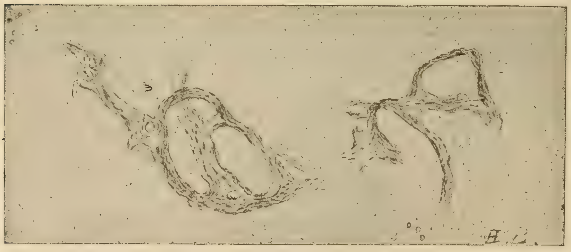

Fig. 7.—Elastic tissue from lung showing alveolar arrangement. × 50.

In judging the significance of blood in the sputum one should always bear in mind its quite numerous possible sources; the nose, the mouth, gums and the pharynx, as well as the bronchi and alveoli.

Elastic tissue (Figs. 6 and 7) is one of the most important constituents found in the sputum. Before the discovery of Bacillus tuberculosis its presence was the best laboratory evidence of pulmonary tuberculosis, although it can also be found in the sputum of cases of pulmonary abscess or gangrene or cancer. It is our best proof of cavity formation due to any destructive disease. In some cases of tuberculosis elastic tissue is found before the tubercle bacilli. The masses of fibers of elastic tissue are often large enough to be visible to the unaided eye. Such is the case in pulmonary abscess and gangrene. In tuberculosis, in which molecular disintegration is the rule, these masses are very small and the most of this tissue is present as single fibers. To find it a little of the sputum is poured on a glass plate about 14 inches square and then pressed out by another glass plate about 6 inches square. The larger plate should rest on a dark background. The particles containing elastic tissue appear as small grayish-yellow dots which are easiest found with a small hand lens. Any suspicious particle may be exposed by sliding the upper glass away, picked up with a needle, crushed on a slide under a cover-glass and examined. It is in these masses also that one would have the best chance of finding tubercle bacilli. Instead of the glass plates for this examination some prefer to use Petri dishes, or wooden sputum boxes painted black, or crockery dishes with the base half black and half white. One must be careful not only to burn or sterilize these utensils after use for safety's sake, but also to cleanse the crockery and glass well in chemicals which will destroy all organic matter (a saturated solution of potassium bichromate in concentrated sulphuric acid is recommended), else there is danger later of finding the tubercle bacilli of a previous examination. Particles of food will confuse the beginner.

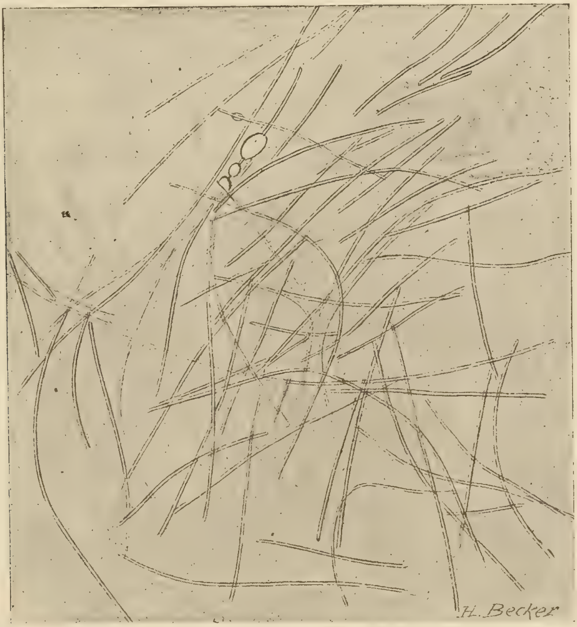

FIG. 8.—Fatty acid crystals resembling elastic tissue in the sputum of a case of bronchiectasis. × 400.

When no fragments containing elastic tissue are found, search should be made under high magnification for single fibers. For this it is well to select the grayish masses (presumably from a tuberculous cavity), or the grass-green or slightly rusty particles (such as are found in subacute caseous pneumonia). Unless the cover-glass is well pressed down to give a very thin preparation the single fibers may be overlooked.

To demonstrate elastic tissue some prefer to destroy all other organic matter. Mix 10 c.c. of sputum with an equal amount of 5 to 10% KOH or NaOH and boil in a porcelain dish until the mass is homogeneous. About 4 volumes of water are then added and the fluid is shaken up and centrifugalized. The value of this method is doubtful since the elastic tissue fibers will have lost their characteristic appearance and appear as pale, swollen threads.

Elastic tissue stains, *e.g.*, Weigert's stain, may be used to demonstrate this tissue in the sputum. Lord recommends the following method: From 0.5 c.c. to 1 c.c. of the thick purulent sputum is placed in a small Erlenmeyer flask and diluted with 15 to 20 volumes of distilled water. From 3 to 5 drops of KOH are added (as small an amount of KOH as possible should be used in order to keep the specific gravity low) and the mixture gently warmed over the Bunsen flame until the sputum is dissolved. The solution is then sedimented by means of the centrifuge, the supernatant fluid decanted and smears made from the small amount of sediment on cover-glasses by means of the platinum loop. These are dried in the air or over the Bunsen flame, fixed by heat and covered with Weigert's elastic tissue stain. A convenient method is to slip the cover-glass into a large test-tube containing enough of the stain to cover it, and to immerse this in the boiling water of a water bath (a free flame cannot be used since the alcohol will ignite) for about 5 minutes. The preparation is decolorized in alcohol (95%), dehydrated with absolute alcohol, cleared with xylol and mounted in balsam. The elastic fibers appear dark blue or almost black.

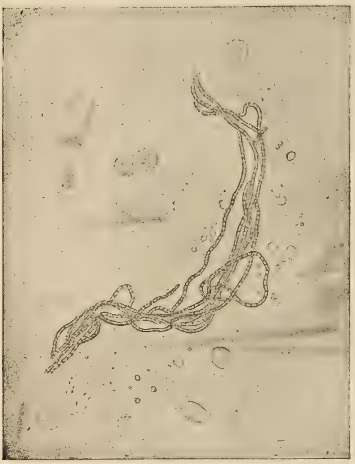

Fig. 9.—A leptothrix form in the sputum, resembling elastic tissue. × 400.

The fibers of elastic tissue in fresh sputum (see Fig. 6) even when single usually may be recognized. In a thin specimen they stand out as very distinct, coarse, sharp, blackish fibers, characterized by their intense refractivity, their wavy outline, sharp edges, an uniform diameter and curling ends. They often branch. They are insoluble in ether and in potassium hydroxide. They are unchanged on warming. Pressure does not produce in them any varicosities. One should not confuse them with fibrous tissue, fatty acid crystals (see Fig. 8), bacteria, or vegetable fibers. The fibers of fibrous tissue appear in bundles of fine, wavy threads which have not the coarse, black, refractive appearance of elastic tissue. (For the fatty acid crystals see page 15.) The chains of bacteria are very confusing, especially certain leptothrix forms (see Fig. 9). These may be found in the fresh sputum, but more often in that which has stood for some time. They have not the same diameter, refractility or wavy outline as elastic tissue fibers and yet the long chains of these organisms sometimes are arranged in a beautiful network which may resemble closely the framework of an alveolus. Under the high power, however, one can make out the difference. Vegetable cells and fibers are as a rule much coarser, are irregular in outline and quite different in appearance. The elastic fibers from the muscle tissue of the food are of the same tissue but as a rule are coarser and more irregular in outline (see Fig. 10). To exclude these the teeth should be well brushed before the specimen is collected. Mould mycelial threads should give no confusion (see page 45).

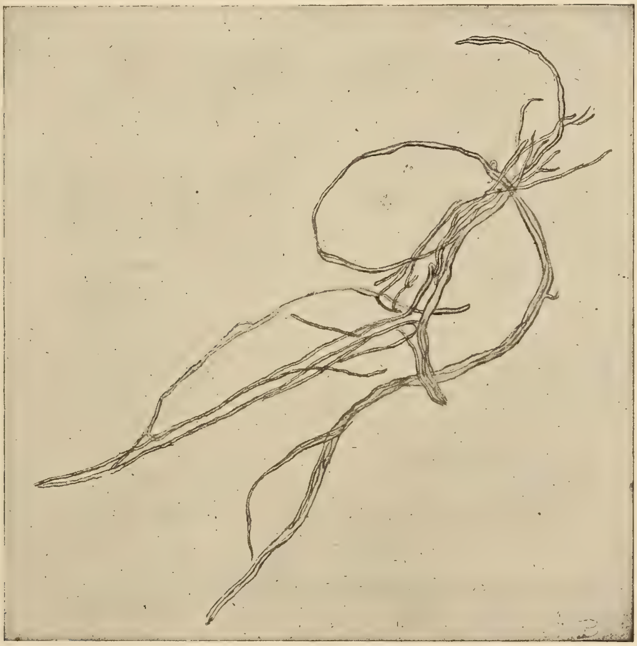

FIG. 10.—Elastic tissue from saliva; origin, the food. × 400.

The presence in the sputum of elastic tissue, apart from that from the food, is proof of disintegration of the lung and, in about 90% of the cases, evidence of tuberculosis. It is found also in gangrene, abscess and neoplasm of the lung.

The fibers of elastic tissue from the lung when in groups may present three arrangements which often suggest their source: fibers from the alveolar walls are long and branching and may preserve the outline of one or several alveoli (see Fig. 7); fibers from the bronchial walls are long and narrow, often fragmented and occur singly or in small groups of two or three clustered closely together in an elongated network; fibers from the walls of arteries appear as sheeting of elastic tissue.

Tissue fragments from ulcers of the larynx contain a coarse network of short, interwoven, elastic fibers.

Crystals are present only in putrid sputum, especially that of putrid bronchitis, bronchiectasis, tuberculosis and gangrene. *Fatty acid crystals* are found in clusters. When very long they may resemble elastic tissue (see Fig. 8) or microörganisms, but they are usually relatively thick, with stiff curves and pointed ends. If pressure is made on them by tapping on the cover-glass varicosities will result. These crystals are soluble in potassium hydroxide and in ether. (The specimen should be dried before ether is added.) If the slide is warmed they will disappear and fat droplets will appear in their place. *Cholesterol crystals* (see Fig. 22) are rare in the sputum and occur usually in connection with fatty acid crystals. *Leucin* and *tyrosin* (see pages 253, 254) may be found in sputum which has decomposed in the air passages and in the contents of a pulmonary abscess. The sheaves of long, black, refractive needles of tyrosin are more easily recognized than are the spherules of leucin. To demonstrate the latter, it is usually necessary to evaporate the sputum. *Triple phosphate* crystals (see page 248) and *calcium oxalate* crystals (see page 250) also occur in putrid sputum. *Hematoidin*, in rhombs or in needles (see page 253), is seen in the sputum in cases of abscess of the lung, of perforating empyema and of a liver abscess discharging through the lung, but seldom after pulmonary hemorrhage in which case the extracellular hemoglobin appears chiefly as amorphous granules.

Charcot-Leyden crystals (see Fig. 74) are long, narrow, diamond-shaped crystals which resemble two very sharp pyramids with their bases together. They have a slight yellowish refractivity and seem quite brittle. They vary greatly in size and are found singly, in groups, or in clusters. They are soluble in hot water, in mineral acids, and in alkalies and stain red with eosin. Viewed in the direction of their long axis one can see that they are hexagonal on cross-section, therefore cannot be identical with Böttcher's spermin crystals, with which they formerly were confused. That these crystals are derived from coarsely granular leucocytes is suggested by the fact that they are found only where these cells are increased, as in the sputum in asthma, in the blood in myelogenous leukemia and in the stools of patients with intestinal parasites. That they are products of decomposition is shown by the increase in their number in a specimen of sputum left for some time in a thermostat.

PLANT PARASITES

The **bacteria,** of which usually there are large numbers in the sputum, are chiefly saprophytes, some from the mouth and others added later by the cup or the air. These multiply very rapidly at room temperature. The *chromogenic organisms* are mentioned on page 5.

To obtain from the sputum the organisms from the lower air passages the patient washes his mouth well, cleanses his teeth thoroughly and then expectorates into a sterile cup. The sputum is at once rinsed several times in sterile physiological salt solution and cultures made. Among these are many saprophytes which flourish in the bronchi in cases of chronic bronchitis, tuberculosis and especially in bronchiectatic cavities. These saprophytes explain the decomposition of stagnant sputum. One finds also pathogenic staphylococci and streptococci which may be primary or secondary invaders of the bronchi. In tuberculosis they aid and perhaps explain the destructive processes in the lungs and cause most of the complications and sequelæ of this disease.

Micrococcus Aureus—Staphylococcus Pyogenes Aureus (Fig. 11).—This organism is a coccus which ordinarily appears in clusters, hence the name "staphylococcus," but when actively growing in tissues usually occur as diplococci. The individual organisms are spheres a little less than 1μ in diameter. It is easily stained in all aniline dyes and is not decolorized by Gram's method. It is not a flagellated coccus. It grows well on all ordinary media. If grown directly from the animal body its colonies will sooner or later develop a golden yellow pigment, seen first at the edges of the thick, glistening, dull white colonies and later spreading throughout the whole growth. The most pigment is produced on potato. This organism liquefies gelatin, produces acid in milk and ferments nearly all sugars with gas production. It is pathogenic to animals, producing either a fatal septicemia—or, if the animal survives for a few days, a pyemia with multiple abscesses. In man it is the common pus-producing organism, causing local foci, as boils, abscesses, etc.

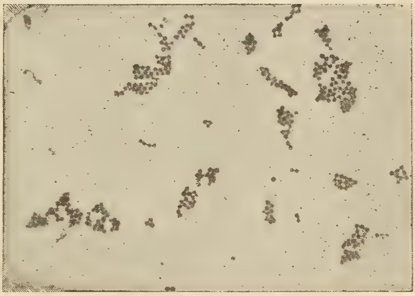

FIG. 11.—Micrococcus aureus. Photomicrograph by Dr. Thomas M. Wright.

Staphylococcus pyogenes albus is also one of the important pyogenic organisms. It differs from Staphylococcus pyogenes aureus only in that it produces no yellow pigment. Many think that it is really a variant strain of aureus.

Staphylococcus epidermis albus is a normal inhabitant of the deep layers of the skin. It is quite similar to Staphylococcus pyogenes albus but is less pathogenic and a feebler grower.

THE STREPTOCOCCUS GROUP.—The single organisms of the chains of streptococci are cocci about 1μ in diameter which grow in chains of from 2 to 100 or more, each slightly flattened against its neighbors.

These chains have no capsule as have the chain forms of Diplococcus lanceolatus and Streptococcus mucosus. They do not decolorize by Gram's method. They grow on all ordinary media, but are feeble growers, forming colonies so minute and translucent that they are easily overlooked and which are quickly overgrown in mixed cultures. In the search for this organism in the urine, sputum, pus, etc., bacterioscopy is often of more value than are cultural methods. There certainly are several varieties of streptococci and yet no classification is quite satisfactory. Some strains do not grow at room temperature; some coagulate milk, others do not; some produce a growth, *i.e.*, a sediment, in liquid media with rather distinctive physical properties; some grow on gelatin, which they do not liquefy, others do not; some hemolyze red blood-cells, others do not; some produce pigment; many differ in their ability to ferment carbohydrates, all are quickly killed off in acid-reacting media, all are insoluble in bile and all decolorize litmus milk. Certain varieties are definite enough to deserve mention.

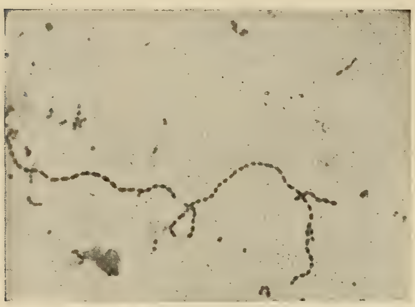

FIG. 12.—Streptococcus pyogenes. Photomicrograph by Dr. Thomas M. Wright.

Streptococcus pyogenes (Fig. 12), formerly almost a "group" name since the other varieties described at the same time were not long accepted, is an intensely pathogenic organism for man, producing rapidly spreading inflammations, *e.g.*, erysipelas, with much necrosis but with little pus production. It is especially prone to cause inflammation of the serous membranes. It is an important cause of septicemia and pyemia. It is the most widely spread of all pathogenic organisms.

Schottmüller, who classified these organisms on the basis of their growth on blood agar (in this technic at least 5% of defibrinated blood should be used and all readings made at the end of 24 hours incubation),[5] designated this as *Streptococcus longus pathogenes seu erysipelatos*, the inciting agent in erysipelas, which produces in blood agar about the colony a wide, clear zone of hemolysis due to an active hemolysin and without methemoglobin formation. Blake would include all those organisms under the name *Streptococcus hemolysans* (or hemolyticus) which produce a true hemolysin. On the basis of complement fixation tests Kinsella and Swift [6] decided that this variety is homogeneous, consisting of members which are nearly identical. Opie [7] found this organism in the mouths of 1 of each 4 or 5 healthy soldiers at Camp Funston. It seemed to play an insignificant part in the production of pneumonia but a very serious part in the causations of complications. It evidently was important in bronchitis. It would seem able to pass through a pneumonic lung and appear in the blood-stream or in the pleural cavity. Lucke, Wight and Kime [8] found at Camps Taylor and Knox that this streptococcus was the organism of a third wave of the "flu" infection, the second "wave" or "crop" being due to pneumococci and non-hemolytic streptococci. Cecil [9] found that 24.7% of the pneumonias of this epidemic were due to streptococci and of these 24% to streptococci of the hemolytic type. The mortality of the streptococcus cases was over 30% while the general mortality for pneumonia was only 15%. Of the streptococcus cases 24.9% developed empyema while of the pneumococcus cases, but 12.9%.

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[5] Blake, Jour. of Med. Research, vol. xxxvi, Mch., 1917.
[6] Jour. of Exp. Med., Aug. 1, 1918, xxviii, p. 169.
[7] Jour. A. M. A., 1919, vol. 72, p. 108.

Streptococcus mitior seu viridans (Schottmüller) produces green colonies in blood agar and changes oxyhemoglobin to methemoglobin without the production of a true hemolysin. It is rarely hemolytic and then only apparently so since the red cells in the clear zone do not lose their structure; but it may form a narrow, clear zone about its green colonies. The name *Streptococcus alpha hemolysans* has been given to these organisms. Krumweide and Valentine [10] considered the viridans not one type but a heterogeneous group. One of these types which does not produce hemolysis nor methemoglobin has been named *Streptococcus saprophyticus* but is classified now as viridans. Blake includes under the term Streptococcus viridans all which transform oxyhemoglobin to methemoglobin and includes also all which produce no change in blood. In this group he recognizes *Streptococcus buccalis* which will ferment lactose but not mannite, *Streptococcus fecalis* which will ferment both and *Streptococcus equinus* which ferments neither. Streptococcus buccalis has been obtained from the urine in acute nephritis, from alveolar abscesses, from the heart valve in endocarditis, from tonsils and mouth, from the sputum in pneumonia and in bronchitis and from the blood in septicemia; Streptococcus fecalis, from the tonsils, from alveolar abscesses, from the urine in acute nephritis and from abscess in pyemia; while Streptococcus equinus, from the tonsil and from the heart valve in endocarditis. On the basis of fermentation reactions Kinsella and Swift [11] assigned the 28 strains they studied to the *fecalis group*, the *mannite fermenters* and the *salivarius group*. The results of their complement fixation tests do not, however, justify this grouping and show the group to be heterogeneous. Holman [12] has given a much more elaborate classification.

Streptococcus mucosus (Schottmüller) is an organism so similar to Micrococcus pneumoniæ that many doubt that it is a separate bacterium and group it with this organism under the name *Pneumococcus mucosus*. It differs from the former 2 varieties also in that it is bile-soluble. The organism, named also *Streptococcus mucosus capsulatus*, was found by Buerger[13] in the cerebrospinal fluid of a fatal case of acute meningitis and in the mouths of 6 normal persons. It is held responsible for some of the epidemics of "influenza," or at least of some of the complications. It is sometimes highly virulent and in human blood it is nearly unsusceptible to phagocytosis. Obtained from the blood and secretions of inoculated mice, it is found to occur chiefly as round, biscuit-shaped, or slightly lancet-shaped, encapsulated cocci, most of them in pairs, but always a few in chains of 4 or even 6, often irregularly arranged. The capsule is wide and easily broken. When this organism grows in pairs it resembles Micrococcus pneumoniæ but the cocci are never as uniformly and definitely lancet-shaped and the capsules are wider and show no traces of the constrictions partially separating the cocci so often seen in the capsules of the organism of pneumonia. Streptococcus mucosus capsulatus grows luxuriantly on serum agar and glucose serum agar. On blood agar it produces a mucoid growth with a dark-green zone. This is a very important point in its identification. It remains encapsulated through many subcultures. The colonies have the same watery, almost transparent, appearance as have those of Micrococcus pneumoniæ, but they grow faster and tend to coalesce, so that the surface of the slant medium is finally covered by the growth.

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[8] Arch. Int. Med., 1919, vol. 24, p. 154.

[9] Jour. A. M. A., 1918, vol. 70, p. 728.

[10] Jour. of Infect. Diseases, 1916, vol. xix, p. 760.

[11] Jour. Exp. Med., June, 1917, xxv, p. 877.

[12] Jour. Med. Research, 1916, xxxiv, 177.

Micrococcus catarrhalis is a common inhabitant of the respiratory passages of persons who are healthy as well as of those who are diseased. It is often seen in the nasal secretion and sputum of patients with common colds, it may be the cause of mild catarrhal inflammations and has been carefully studied in connection with epidemics of influenza. When this organism occurs as biscuit-shaped diplococci, and especially when intracellular, as is so often the case, it closely resembles in morphology the Gonococcus and Meningococcus intracellularis. It grows easily, however, on all simple culture media, which the gonococcus does not. Some strains so resemble the meningococcus that special media are required to differentiate them. For this purpose Dunham proposed a medium of sheep serum containing 1% of glucose. On this medium the meningococcus produces acid in 24 hours but no coagulation, while Micrococcus catarrhalis produces either acid and coagulation or an alkaline reaction. When Micrococcus catarrhalis grows in chains it resembles Streptococcus pyogenes, except that the individual cocci are larger and can be decolorized by Gram's method. Most recent writers (*e.g.*, Holt) consider this an organism unworthy of serious attention. It has never been isolated from the blood or known to cause a general pyemic infection (Hasting and Boehm).

Bacillus Tuberculosis (Fig. 13).—Possibly on no single clinical test is as much human interest centered as on the search for Bacillus tuberculosis in the sputum. A few drops of sputum are dried and stained in a certain manner. If in this specimen the observer finds one rod of a certain color and shape he has in the past been willing on this evidence alone to insist on an entire reorganization of the patient's life and to condemn him to exile from home for months or even years. Fortunately within the past few years wiser counsel has prevailed and fewer doctors rest so momentous a question on the stained specimen alone. Some have made it a practice to send the sputum of each of their patients with a cough to the State laboratory and make their diagnosis from these reports. The laboratory man can help the physician make his diagnoses but is not in a position to make them for him. One should in each case interpret the laboratory report in terms of the clinical history and the physical examination.

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[13] Mt. Sinai Hosp. Reports, 1907, Centralbt. f. Bakt. Orig., vol. 41, p. 314

Among the errors inherent in this test are the following: First, the specimens may have become wrongly labelled.

Second, a red bacillus in the stained specimen with almost correct morphology may not be Bacillus tuberculosis; it may be some other acid-fast organism from the nose or mouth, from food, milk or water.

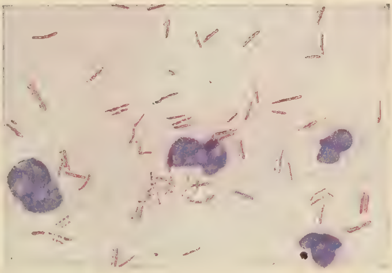

Fig. 13.—Tubercle bacilli, stained with carbolfuchsin and decolorized with nitric acid. × 900.

Third, this bacillus may be Bacillus tuberculosis but not from the patient under consideration. The dust of the air which settles in his sputum cup often includes this germ; the sputum cup may not have been cleaned perfectly (it may have been sterilized properly, but that is another matter) and so we find in the sputum bacilli from some other patient. The same danger applies to the hatpin we use in spreading the specimen, to the glass plates on which we pour it, and especially to the slide and cover-glass on which the specimen is made.

Fourth, the tubercle bacillus we find may have come from the sputum of our patient and yet he may not have tuberculosis; he may have inhaled dust containing dead or living tubercle bacilli and these we find in his sputum.

The above mistakes are to be feared in case one or a few bacilli are found on a single examination. Therefore it should be an invariable rule to confirm a positive report at least once, taking every precaution to rule out all chances of such errors (see page 59).

On the other hand, a negative report may lead to error. For instance, in the sputum of from 60 to 75% of early cases of tuberculosis no tubercle bacilli can be found; or, we may not have selected for examination the proper portion of sputum; and, lastly, the bacilli may not take their specific stain. In case of doubt the organism in question should be grown on media or injected into a guinea-pig (see page 27). And yet so valuable is this clinical laboratory test that notwithstanding all these possible errors the test is one of the most valuable we have. The routine of this examination is as follows:

The patient should at night cleanse his mouth and teeth and the next morning expectorate the first sputum he can raise into a perfectly clean cup. The sputum is then spread on a glass plate and the caseous particles already mentioned should, if present, be selected for the examination, but if none are found, smears should be made from the small bloody or purulent masses. The bacilli may be found in the masses of blood of the initial hemoptysis. In advanced cases, bacilli may occur in goodly numbers in practically all portions of the watery mucoid as well as of the mucopurulent sputum.

In case but very few bacilli are present the sputum should be spread on a large glass, squeezed under a smaller glass and careful search made with a hand lens for proper particles for examination. This is the best method we have. Others prefer to render the sputum homogeneous and fluid and then either to precipitate the bacilli to the bottom of the tube or to salt them to the top.

The best quick way of obtaining a sputum sufficiently homogeneous and fluid is Löffler's [14] modification of *Uhlenhuth's antiformin method.* Antiformin is supposed to destroy all bacteria except those which are acid-fast and all other formed elements as well. A measured quantity of sputum (5, 10 or 20 c.c.) is mixed in a Jena flask with an equal quantity of 50% antiformin (which contains $NaClO$ and $NaOH$) and boiled not over 15 minutes. The solution will foam considerably and become somewhat brownish in color. To each 10 c.c. of the fluid are now added 1.5 c.c. of a mixture of chloroform (1 part) and alcohol (9 parts). The whole is shaken vigorously until a fine emulsion is produced and then some of this emulsion is centrifugalized for 15 minutes. The heavier constituents, including some of the bacilli, collect in a film just above the chloroform. The supernatant fluid is poured off, the film is transferred to a cover-glass, or better, directly to the glass slide, and the excess of fluid is absorbed by filter paper. A drop of egg albumin mixed with carbolic acid (enough to give a 0.5% solution), or, better still, some of the original sputum not treated with antiformin, is added to fix the sediment to the slide. This sediment thus prepared is spread by a second slide or a clean hatpin into a thin smear, allowed to dry in the air, fixed in the flame, and stained. No cover-glass is necessary. The cedar oil is put directly on the stained smear. One can spread the smear more easily by holding the slide at such distance above a flame that it is slightly warmed.

Another method is that of Paterson, who adds 2.5 c.c. of antiformin to each 10 c.c. of sputum and as soon as the sputum is dissolved pours the resulting fluid into centrifuge tubes. These centrifugal tubes when not in use are kept in a jar of potassium bichromate and sulphuric acid, and are well rinsed with distilled water before using. The tubes are stoppered with new corks, shaken, and allowed to stand 24 hours at room temperature or from 4 to 6 hours in a thermostat at 30° C. The tubes are then again shaken and centrifugalized, the supernatant fluid poured off, the tubes refilled with sterile physiological salt solution, shaken and again centrifugalized. This is repeated a second time. The sediment is then transferred to a slide and treated as above.

One attempting to centrifugalize bacteria suspended in a fluid should remember that the reason why we so often succeed is that many of the organisms are attached to small masses of sediment which are heavy enough to be thrown to the point of the tube. The specific gravity of tubercle bacilli lies roughly between 1.010 and 1.080, and if, as often happens, the specific gravity of the fluid is heavier than that of the bacilli (the specific gravity of sputum varies from 0.929 to 1.2242) centrifugalizing will send the most of the organisms to the top rather than to the bottom of the tube. In case they are suspended in a fluid not coagulable by alcohol one can prevent this by diluting the fluid with an equal volume of alcohol. The mixture is then so light that even the free bacilli will sink to the bottom. Others prefer to add to the fluid an equal volume of a 25% NaCl solution and allow the mixture to stand 24 hours. In this heavy liquid the bacilli will rise to the top.

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[14] Deut. med. Wchs., Oct. 27, 1910, vol. xxxvi, No. 43, p. 1987.

Staining.—The possibility of an almost specific stain for Bacillus tuberculosis depends on the fact that this organism resists decolorization by acid and by alcohol.

As a routine method we recommend the following technic which we shall describe first briefly, then in detail. The specimen, dried and fixed by heat on a slide, is covered with the Ziehl-Neelsen carbolfuchsin solution and heated over a small flame. Fresh stain is from time to time added to keep the specimen from drying and the glass from cracking. The staining solution covering the smear should actually boil for at least 1 minute. The stain is then poured off, the smear washed in water and dried with a blotter. It is then decolorized with acid alcohol until it will decolorize no more. Next, it is washed in water, dried with a blotter and covered for about 5 seconds with Löffler's methylene blue. After the excess of this stain is washed off the specimen is again dried with a blotter and mounted. On examination the tubercle bacilli will be found stained red and all other organisms blue.

The following points of technic deserve more detailed statement. Bacillus tuberculosis is stained with difficulty but when once stained is also decolorized with difficulty. The entire specimen is therefore first overstained with a very penetrating dye which will certainly stain Bacillus tuberculosis. This will of course easily overstain all other bacteria. The specimen is then decolorized with a reagent which will remove the stain from practically all organisms except Bacillus tuberculosis, so that when counterstained with a blue or brown dye the tubercle bacillus alone will retain the original red stain.

Ziehl-Neelsen's carbolfuchsin mixture is the stain in common use for this purpose (Fuchsin 1 gm.; absolute alcohol 10 c.c.; and 5% carbolic acid 10 c.c.) Bacillus tuberculosis usually can be deeply stained in 1 minute in this solution at the boiling temperature, or in 24 hours in the cold stain. By the rapid method the carbolfuchsin solution should actually boil for at least 1 minute, but 1 to 4 minutes are safer or the tubercle bacilli may not take the stain. Some prefer to submerge the slide or cover-glass in a flat dish full of the stain and to boil this over a free flame. Mere steaming is not sufficient. Even with the best of technic probably not all of the tubercle bacilli in the specimen will take any stain.

The slow method is better and should be used if haste is not necessary since the specimens are much better stained. Heat certainly injures the specimens. The slides are placed vertically (to avoid a precipitate on the specimen) in a tall staining jar full of the carbolfuchsin solution and allowed to stand for 24 hours at room temperature (or in a thermostat). The further steps are those described above.

Having stained deeply as many of the bacilli in the specimen as possible, the next step is to decolorize all but the tubercle bacilli. The best reagent for this purpose is *acid alcohol* (2% HCl, some say 3%, in 80% alcohol). The slide may be dipped into a vessel of this acid alcohol, or this fluid may be repeatedly poured on and drained off the slide until the smear is almost colorless. A little time may be saved by gently warming the slide during this process. Some decolorize under the low power of the microscope and are able to determine very accurately when the end point is reached. Another decolorizing fluid is 25% nitric acid, and still another is the very popular Gabbett's fluid which contains 25% sulphuric acid (see below). Gabbett's fluid, however, "burns" the specimen more than does nitric acid, and as a result the bacilli look too thick and are less distinctly beaded. Acid alcohol is far preferable to either sulphuric or nitric acids in aqueous solution since there is a large group of acid-fast organisms which may be confused with Bacillus tuberculosis but very few which are acid-alcohol-fast.

After the specimen is decolorized it is washed in water and blotted with filter paper. If as a result any red tint returns, the acid is again applied.

As counterstain Löffler's methylene blue (see page 38) is in general use. Löffler himself, however, used a 0.1% solution of malachite green (Malachitgrün crystallen Chlorzinkdoppelsalz, Hechst). The decolorized, washed and dried specimen is covered with this stain for about 5 seconds, the stain then washed off with water and the specimen mounted. The very popular *Gabbett's fluid*, in which is not only the decolorizing agent but also the counterstain, contains enough methylene blue (1 to 2 gms.) to saturate 100 c.c. of 25% sulphuric acid. By this method the specimen, after the excess of carbolfuchsin has been washed off, is dried and then covered for 1 to 5 minutes with Gabbett's methylene blue. This is then washed off in water. If any pink tint remains, except in the thick portions, the smear is again covered with Gabbett's solution and again washed. This method is very popular since so easy. It is fairly satisfactory for the examination of those specimens in which tubercle bacilli are practically the only acid-fast bacilli to be found and especially if repeated examinations are to be made in cases the diagnosis of which is not doubtful in order to determine any increase or decrease in the number of bacilli present.

Not all tubercle bacilli are acid-fast. This probably explains our failure to find them in the sputum of certain cases of proven tuberculosis. Since those tubercle bacilli which are not acid-fast will not decolorize by Gram's method, Much recommends that we search for Gram positive bacilli in the sputum in which we fail to find acid-fast organisms.

It is also true that not all acid-fast rods seen in specimens are tubercle bacilli. Doubtless far too many specimens called positive should have been reported as negative. Some laboratory workers estimate that this error is present in about 10% of the positive reports made.

Morphology.—The majority of tubercle bacilli found in the sputum are from 1.5 to 3.5μ long and about 0.2μ wide. Some, however, are over 11μ long, branch, are curved and resemble spirochetæ. Many, even of the shortest, are somewhat bent. Chains of these bacilli are sometimes seen.

In stained specimens the bacilli are found scattered or in clumps and those in clumps may lie parallel or crossed. Beaded forms (from one to eight beads in each rod) resembling streptococci are common. These are considered degenerated forms. That the young bacilli stain less intensely than do the older forms is now disputed.

Much's granules,[15] minute acid-alcohol-fast granules, are considered to be fragments of tubercle bacilli. They are often found together with perfect tubercle bacilli in specimens which have been treated with antiformin while in about 10% of the cases of tuberculosis one finds them and no perfect bacilli.

Bacillus tuberculosis is not a spore-bearing organism and is as susceptible to heat as are the other bacteria which do not produce spores;[16] *i.e.*, all are killed in twenty minutes at 60° C.

Tubercle bacilli will retain their virulence in dried sputum for from 3 to 10 months.

While other bacteria contain only from 1.7 to 10% of fatty matter, tubercle bacilli contain from 10 to 37%. This may explain their unusual staining characteristics.

Animal Inoculation.—The theoretically best method of identifying tubercle bacilli is by inoculating guinea-pigs with the sputum under examination. This never has been a popular method because Diplococcus lanceolatus is often present also and would kill the animal, and also because one must wait from 4 to 6 weeks for a result. These objections are not insuperable, however, for the sputum can be freed from the throat and mouth organisms by washing it (see page 19); also, since dead tubercle bacilli will produce tubercles, all organisms present may be killed by heat and then the sputum injected into a guinea-pig. To shorten the time one must wait for a result it was suggested by the work of Murphy and Ellis [17] to expose the guinea-pig to massive doses of Röntgen rays which will inhibit the protecting action of the lymphatic tissue.

This technic has been standardized by Eckford [18] as follows: The sputum is digested for 15 minutes with 4% potassium hydroxide after Petroff's method and is centrifugalized and washed before injection. (If the material to be tested is not sputum but of a fibrinous nature it is digested in 15% antiformin until fluid. If it is a clear fluid it is merely centrifugalized.)

From 0.5 to 1 c.c. of the material is injected both into the groin and into the abdomen of the animal, which is then exposed every second day to a five-minute dose of X-ray for at least 3 exposures. (The dose is given with the Coolidge tube, the current 60,000 volts, the spark gap 6 inches, the filter 0.5 mm. of aluminum and the target 12 inches.) If a nodule appears and is still present in 2 weeks it is removed under a local anesthetic, smeared and examined. If a positive diagnosis cannot be made the animal is kept for at least 6 weeks and then examined.

The Cultivation of Bacillus Tuberculosis.—Some of the best workers cultivate the organism for clinical diagnosis.

Petroff's Medium.[19]—Petroff proposed for the clinical demonstration of Bacillus tuberculosis a medium which contains:

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[15] Korber, Deut. med. Wchschr., August 8, 1912.
[16] Rosenau, Bull. of Hygienic Lab., Sept., 1909, No. 57.
[17] Jour. of Exp. Med., 1914, xx, 397.
[18] Jour. of Lab. and Clin. Med., 1917, ii, 175.
[19] Jour. of Exp. Med., 1915, xxi, 38.

Egg (white and yolk), 2 parts by volume.

Meat juice, 1 part by volume.

Gentian violet, 1% alcoholic solution, enough to make a solution of 1 : 10,000.

To make the meat juice, 500 gms. of beef or veal are infused in 500 c.c. of a 15% solution of glycerin in water. After standing for 24 hours the meat is squeezed in a sterile meat press and the juices collected in a sterile beaker.

The shells of the eggs used are sterilized by immersing them for 10 minutes in 70% alcohol or by pouring hot water over them. They are then broken into a sterile beaker well mixed and filtered through sterile gauze.

About 3 c.c. of this medium are poured into each sterile tube and dried for 3 successive days: on the first day at 85° C. until the medium is solid and on the second and third days for not more than 1 hour at 75° C.

Under the most favorable conditions it takes at least 6 days for a single tubercle bacillus to grow to a visible colony.

In sputum examination about 5 c.c. of fresh sputum and an equal amount of 3% NaOH are well shaken and left in the incubator for 20 or 30 minutes until the sputum is fairly well digested. It is then made neutral to sterile litmus paper with *0.1N* HCl, centrifugalized and the sediment inoculated into several test-tubes containing the above-described medium.

Petroff obtained very uniformly satisfactory results. The cultures usually are pure since the NaOH and later the gentian violet should kill off all other bacteria.

Bacillus tuberculosis grows better the longer it has been artificially cultivated in the laboratory.

That this method cannot supplant the other methods is clear from the fact that a few strains have been found which fail to grow well on any medium.[20]

BOVINE TUBERCULOSIS.—It is now agreed that the bacillus of human tuberculosis and that of bovine tuberculosis are distinct types. Both are pathogenic for man, the bovine especially during infancy and early youth.

Differentiation Between the Human and Bovine Types of Bacillus Tuberculosis.—The method described is that followed by Fraser[21] who employs tests described by Theobald Smith. In case the bacillus in question is to be isolated from diseased tissue this material itself is injected beneath the skin of guinea-pigs. In this way a pure culture of Bacillus tuberculosis may be obtained and the possible strains of saprophytic tubercle bacilli which may flourish as secondary invaders in lesions caused by more pathogenic organisms, and which grow on media more readily than the latter, are excluded. The inoculated guinea-pigs are permitted to live from 4 to 6 weeks, during which time careful records are kept of their condition. They are then killed and cultures from the tuberculous organs, especially the glands and spleen, are made by rubbing with a small platinum spud the diseased material onto the surface of the media. The tubes are sealed with paraffin. The media used are numerous. Theobald Smith[22] used dog's serum; Hiss and Zimmer recommend slants of agar to which rabbit's blood has been added, 1 to 2 c.c. to each tube; glycerin agar (containing 3 to 6% of glycerin) is also used. In about 10 days a growth is usually apparent. A rapidly growing organism is likely to be human in type, while one which gives a weak and scanty growth is probably bovine. This test alone is not conclusive.

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[20] Corper, Am. Rev. of Tuberc., 1919, iii, 461.
[21] Jour. Exp. Med., October 1, 1912, vol. xvi, p. 432.
[22] Jour. Exp. Med., 1898, vol. iii, p. 451.

If in the early and original culture the bacilli are long, slender, slightly curved and regular in shape and show granules, and if they tend in later cultures to grow in still longer forms the chances are that they are of the human type; if they are thick and short, from 1 to 1.5μ in length, straight, not regular in shape, but some of them are spindles, others broader at one end, the bovine type is suggested. This test also is not conclusive.

Glycerin inhibits the growth of the bovine, but stimulates that of the human, type. For this reason if a primary culture on glycerin medium grows luxuriantly from the beginning it is without much doubt the human type; if sparsely or not at all, the bovine. (The medium used in this test is glycerin bouillon made of beef or veal with peptone, 1%, glycerin, 6%, and rendered slightly alkaline. This is a very valuable test.)

The "change of reaction" test proposed by Theobald Smith is almost conclusive. The main difficulty in this test lies in obtaining a suitable surface pellicular growth of the organism in question. To get this the organism is first cultivated on a tube of egg medium with a small quantity of glycerin bouillon in the bottom of the tube. The pellicle which extends over the surface of the bouillon is then transferred to an Erlenmeyer flask containing the test medium. This medium is glycerin bouillon originally neutral to litmus which must then be carefully titrated and brought up to standard acidity by adding 0.05 c.c. of $0.2N$ HCl. As the organism grows on this standardized medium and the pellicle develops, the acidity of the medium in the case of the human type increases, while in the case of the bovine type the acidity for a time diminishes and the medium may even become alkaline. To determine the change of reaction, 5 c.c. of the fluid are removed every 10 days, diluted to $\frac{1}{10}$ its strength and titrated hot.

The inoculation test is very valuable, perhaps absolute. If a rabbit weighing on an average 2000 gms. is inoculated intravenously with a known amount of the human bacillus (Fraser recommends 0.01 mg. of dried weighed pellicle emulsified and the emulsion so diluted that 1 c.c. contains 0.01 mg.) the resulting lesions will be few and small and after a time show a tendency to undergo retrogression. Death as a rule occurs in 6 months or longer and then often not from tuberculosis. If a bovine bacillus is used an acute disseminated rapidly fatal tuberculosis will develop.

The *bacillus of fish tuberculosis* is similar in morphology to Bacillus tuberculosis and is rather acid-fast but grows at surprisingly low temperatures (15° to 30° C.).

The *bacillus of avian tuberculosis* closely resembles Bacillus tuberculosis in its morphology and staining characteristics. It grows on artificial media much more rapidly than does the human type and at a temperature from 41° to 45° C., which is above that at which the latter will thrive (40° C.). There is evidence that either of these organisms may by proper cultivation and proper passage through animals be made to resemble the other.

Acid-fast Organisms.—The important group of acid-fast organisms deserves careful study. Supposed at first to be peculiar to but few bacteria, resistance to decolorization by acid is now known to characterize a large group, chiefly of non-parasitic organisms. Among these are [23] Bacillus tuberculosis, Bacillus lepræ, the smegma group, the "milk and butter" bacilli (Rabinowitch), the timothy hay bacillus and the grass bacillus of Moeller, bacilli found in manure (Moeller), and a large group found in sewer water, in soil, etc. The majority of these organisms are of much greater interest to the hygienist than to the clinical microscopist, altogether the interest aroused by the report that it is easy to find Bacillus tuberculosis in the circulating blood of consumptives (Rosenberger) demonstrated to the microscopist the necessity of examining his reagents, even distilled water, for acid-fast bacilli.[24] Several of these organisms have considerable clinical importance, as they are often mistaken for Bacillus tuberculosis. Chief of these are the smegma organisms which are found in those parts of the body where the secretions of the skin are allowed to collect, as around the genitals (where they were first discovered, whence their name, and where they abound) within the folds of the thighs and buttocks, in the axillæ, in cerumen, etc. The smegma bacilli while acid-fast are not alcohol-fast and when grown in culture media many are not even acid-fast. It may be that the difficulty in decolorizing them by acids and the ease with which they are decolorized in acid alcohol are due to a fatty coating gained from the secretion of the skin where they were found and not to any quality of their own. Some strains of the smegma bacilli have a morphology identical with that of Bacillus tuberculosis, but in general they vary much more in size and appearance than does the latter. Acid-fast organisms, probably of the same group as smegma bacilli, are found also in the nose, in the coating of the tongue, in the tonsillar crypts, in the throat, in the stools, in the sputum, and in destructive diseases of the lungs, especially pulmonary gangrene. They are grown with difficulty, at first on media rich in human blood-serum or hydrocele fluid, but the later subcultures grow readily on glucose agar.

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[23] Borrel, Bull. Inst. Pasteur, May 30, 1904.

These non-pathogenic acid-fast bacilli do not constitute a biological group. Indeed, their ability to withstand decolorization by acid is almost the only characteristic they have in common. While most of those we find in clinical examinations are shorter, thicker, and more homogeneously stained than Bacillus tuberculosis, yet some have exactly the same morphology. Practically all are decolorized by alcohol and most of them are not particularly acid-fast. It is to be borne in mind, however, that, as in the case of the smegma bacillus, the text book descriptions of these non-pathogenic acid-fast organisms are based on studies of the organisms as grown on artificial media, rather than as found in nature, while we study Bacillus tuberculosis not in cultures but as obtained directly from the host. Since the smegma bacilli grown on artificial media lose some or all of their acid-fast properties, it is possible that, should we obtain the other organisms in the same way in which we obtain the tubercle bacilli, *i.e.*, immediately from the host, we might find them much more acid-fast than we now think and it is possible that they are a greater source of error than we realize. According to our present knowledge, however, they all are decolorized by alcohol and so this method should be our routine in searching for Bacillus tuberculosis.

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[24] Burville-Holmes, Am. Jour. of Med. Sci., 1910, vol. xxxix, p. 99.

Several[25] organisms of pseudo-tuberculosis which produce pathological lesions very similar to those of tuberculosis have recently been described, but none of these organisms themselves could be confused with Bacillus tuberculosis, so different is their morphology.

Bacillus Lepræ.—Bacillus lepræ resembles Bacillus tuberculosis in that it is alcohol- and acid-fast. Morphologically it is similar although slightly more slender. Some claim that it can be cultivated, although with extreme difficulty, on media containing glycerin, and that the bacillus obtained from cultures is no longer acid-fast, but the chances are that no organism yet cultivated was Bacillus lepræ.

In cases of leprosy, Bacillus lepræ is present in large numbers in the naso-pharyngeal, urethral, and vaginal secretions, in the saliva, and in the feces. Since the nasal lesions are among the earliest in leprosy and since it is easy to find this organism in the nasal secretion thus the early and satisfactory diagnosis of leprosy, is possible. Smears from these secretions are prepared and stained just as for tubercle bacilli, always using acid alcohol as the decolorizing agent.

B. D., No. 3990, aged 59, admitted December 10, 1916, for destructive-lesions of fingers and toes for years supposed to be luetic, was stopped in the examining room by two internes who suspected it was leprosy and confirmed their suspicions before the visiting physician could arrive by smears of the nasal secretion, which showed remarkable numbers of Bacillus lepræ.

Streptothrix Pseudo-tuberculosa (*Streptothrix eppingeri*).—This streptothrix was first found by Flexner[26] in the lungs of a patient with symptoms suggesting pulmonary tuberculosis. Warthin and Olney[27] collected five such cases. While this streptothrix may be the primary invading organism it is oftener part of a mixed infection, as in cases of tuberculosis with cavity formation and of bronchiectasis. This organism grows in true branching threads which form large, entangled masses, even grossly visible as minutely grayish granules in a white, homogeneous, not bloody sputum. Some of the filaments are very long and thick, with short branches and without club-shaped ends. They are acid-fast, the carbolfuchsin giving them a beaded appearance, but they are slowly decolorized by 95% alcohol and by 30% nitric acid. They often resist decolorization by Gabbett's stain. They stain by Gram's method. The streptothrix group is discussed by Claypole.[28] These organisms (Lord) usually grow readily but slowly on all culture media under aërobic conditions and at room as well as body temperature. Cultural peculiarities are variable. Under the microscope young colonies usually show the presence at the periphery of radially arranged and branching filaments. In smears from cultures rods with conical extremities may be observed as well as the slender branching filaments. Bacillary and coccus-like forms may arise in fresh material as well as in older cultures by the breaking up of the filaments. A surface growth may be seen on bouillon or the formation of ball-like masses at the bottom of the tube without clouding of the medium. The results of animal experiments are inconstant. In some instances local abscesses follow subcutaneous or intraperitoneal injection, while widely disseminated, yellowish, miliary tubercle-like nodules may follow intravenous inoculation. The nodules on histologic examination are infiltrated with leucocytes and have a more or less extensive central necrosis. Spore formation (chain sporulation) is thought to occur.

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[25] Abbott and Gildersleeve, Centralbl. f. Bakt., 1902, xxxi, p. 547.
[26] Johns Hopkins Hosp. Bull., June, 1897.
[27] Amer. Jour. Med. Sci., 1904, vol. cxxviii.
[28] Jour. of Exp. Med., Jan. 1, 1913, vol. xvii, p. 99.

The sputum in pulmonary streptothrix infection is more or less abundant, purulent and at times streaked with blood. Cases with hemoptysis have been reported. In other cases the clinical features are those of empyema and, if the chest wall is perforated, suggest actinomycosis, while in still other cases the course resembles that of a pyemia.

The leptothrix group of normal mouth organisms may flourish in abundance in the lungs, especially in putrid gangrenous disease. Their probable effect is to aid in the decomposition of the sputum. Miller has separated from the group formerly called "Leptothrix buccalis" several organisms among which are Leptothrix innominata, an organism which is unsegmented, straight, but sometimes wavy, and from 0.5 to 0.8μ broad. One always finds it in the tartar of teeth. This organism cannot be cultivated and is stained a pale yellow by iodine solutions. Bacillus buccalis maximus is an organism from 30 to 150μ long and 1 to 1.3μ broad arranged in long single threads or in bunches of parallel threads. It takes a deep blue stain with iodine. It cannot be cultivated. Leptothrix maximus buccalis is an organism somewhat longer than the last mentioned but otherwise similar except that it does not give the iodine reaction. For the mouth spirochetæ, see page 41.

MICROCOCCUS PNEUMONIÆ is a small, oval coccus, about 1μ in longest diameter and usually arranged in pairs but often in chains. Even when in chains one can usually see that the individuals are oval, their long diameters in the line of the chain. The free ends of the diplococci are often pointed like a lancet (or better still, a candle flame), hence the former name, "Diplococcus lanceolatus." Micrococcus pneumoniæ is a capsulated organism whether in pairs or chains. There is always doubt about the identity of any non-capsulated diplococcus.

Among the *capsule stains* the following are to be recommended. In all the important point is to avoid the use of pure water.

Welch's Method.—The film, made from the fresh sputum on a glass slide, air-dried and then passed through a flame slowly three times, is first covered with glacial acetic acid for 5 seconds. The excess of the acid is then soaked up with filter paper and the rest washed off with aniline-water-gentian-violet (see page 281) renewing the stain repeatedly until all the acetic acid is removed. This stain is left on for about 3 minutes. The film is now washed in an 0.85 to 2% aqueous solution of sodium chloride until the stain is washed off. The specimen is examined at once in this fluid.

Very fair capsule stains may also be obtained by simply staining the smear for about 30 seconds with the ordinary aqueous gentian-violet solution (5 c.c. sat. alc. sol. to 95 c.c. of distilled water), then washing with a 1% potassium carbonate solution and studying the specimen in this fluid.

Buerger's Method.[29]—The spread, before it is completely dry, is covered with Zenker's fluid minus acetic acid (bichromate of potassium, 2.5 gms.; sodium sulphate, 1.0 gm.; water, 100 c.c.; bichloride of mercury till the fluid is saturated, *i.e.*, about 5%) and gently warmed over a small flame for from 3 to 5 seconds. It is then washed rapidly in water, flushed once or twice with alcohol (95%) and covered for from 30 to 60 seconds or longer with tincture of iodine (about 7%). The specimen is next washed with alcohol (to remove the iodine) until the alcohol remains clear and then the specimen is dried in the air. It is then stained for from 3 to 5 seconds with freshly prepared aniline-oil-gentian-violet (aniline oil 10; water 100; this mixture is shaken and filtered and to the filtrate are added 5 c.c. of saturated alcohol solution of gentian violet). The excess of stain is removed with a 2% NaCl solution. The spread is examined in this fluid.

For methods of cultivating Micrococcus pneumoniæ the reader is referred to Buerger's article.[30] In clinical work the mass of the sputum to be examined may be washed in several changes of sterile salt solution to free it of adherent mucus and then smeared on the surface of slants of Löffler's blood-serum. From this tube at the time of inoculation other tubes should be inoculated in sequence to secure some isolated colonies. It is seldom necessary to make more than 3 or 4 such dilutions. The rabbit and mouse are very susceptible to this organism, the latter especially. Its virulence is tested by inoculating a mouse with fresh sputum. With virulent organisms and a susceptible animal the pneumococcus can be recovered from the heart's blood and the spleen. This is by far the quickest and surest method of identifying this organism. A little of the sputum (or culture, or pleural fluid, etc.) is injected either subcutaneously or within the peritoneum of a mouse or rabbit. If this organism is present the animal will die in from 24 to 48 hours of septicemia and in smears of the beast's blood these encapsulated diplococci can be found in abundance. Considerable variation in virulence will be noted.

Cole has shown that the organism formerly called Micrococcus pneumoniæ (Diplococcus lanceolatus, Diplococcus pneumoniæ) is in reality a group of organisms consisting of at least 4 types which can be separated by biological tests. Their relative frequency and virulence are best illustrated in the following table:[31]

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[29] Report of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Dept. of Health of the City of New York; Part I, Studies on the Pneumococcus, 1905.

[30] The Journal of Experimental Medicine, 1905, vol. vii, No. 5.

	Percentage incidence	Mortality of cases not specifically treated	Found in the mouths of healthy persons
Pneumococcus, Type I	33%	25%	0.8%
Pneumococcus, Type II	33.5%	29%	18.2%
Pneumococcus, Type III (Pneumococcus mucosus)	13%	45%	28%
Pneumococcus, Type IV	20%	12.5% Sometimes 6%	52.9%

The organisms of Type I are alike in so far as their immunity reactions are concerned. For this type an antiserum has been obtained, the use of which has reduced the mortality of cases thus treated from 25 to 8%. Type II also is a fixed type but the serum for the treatment of the cases with this infection is not nearly as successful. Pneumococcus mucosus (Type III) is a far more virulent organism against which no serum has as yet been obtained. This organism has larger capsules than do those of these other groups and forms a sticky exudate in animals. These three types are fixed types. Type IV is composed of organisms which have no common immunity reactions and which are much less virulent. The pneumococci found in the mouths of from 80 to 90% of normal persons are usually of this type.

For the rapid isolation of the organism from the sputum for agglutination tests to determine the type, a mass of the sputum is washed several times in sterile salt solution, rubbed up in a mortar with one-half a cubic centimeter of bouillon and injected into the peritoneal cavity of a mouse. Six hours later some of the peritoneal fluid is removed, or after about 8 hours the animal is killed and the peritoneal exudate washed out with 5 to 6 c.c. of salt solution or bouillon. This suspension of bacteria and leucocytes is then centrifuged at a rate sufficient to throw down the leucocytes. The supernatant fluid is withdrawn and centrifuged rapidly to collect the bacteria. The dense emulsion of bacteria thus obtained may be used for the agglutination tests. These are made by determining whether or not Serum I or II will agglutinate the organisms found. Often the type may in this way be determined at once.

FRIEDLÄNDER'S BACILLUS.—This organism, known also as *Bacillus mucosus capsulatus* appears in the sputum and blood as a short, rather thick, encapsulated, non-motile bacillus with rounded ends. It occurs single, in pairs or in short chains. It does not produce spores. It is decolorized by Gram's method. It grows on ordinary media. Injected into laboratory animals it produces a fatal septicemia.

BACILLUS INFLUENZÆ (see Fig. 14) is one of the smallest of the microscopically visible bacteria. It is a short, slender, non-motile bacillus, with rounded ends, and has a tendency to grow into filamentous forms. It stains faintly and usually has a marked tendency to polar staining, so that it often resembles a diplococcus. It is decolorized by Gram's method and grows only on media containing hemoglobin. The most profuse growth is obtained if pigeon's blood is used. Its growth and virulence are increased if it is grown with other organisms. In the sputum it occurs free or in groups which are often large, while others are found in leucocytes. (Some think that increased phagocytosis is a sign of improvement.) Bacillus influenzæ is sometimes virulent to animals (specially the guinea-pig and small rabbits) but often is not.

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[31] Trans. of the Assoc. of American Physicians, 1915, vol. xxx, p. 234.

The ability to recognize this organism in the sputum depends chiefly on one's familiarity with its morphology. A good method of staining the specimen is as follows: the fixed smear is stained with aniline-oil-gentian-violet (Sterling's) for 1½ minutes and washed in water; covered with Gram's solution 1½ minutes and again washed in water; immersed in 95% alcohol 5 minutes and washed in water; covered with 0.2% aqueous Bismarck brown (20 c.c. of saturated alcoholic solution of Bismarck brown diluted with 80 c.c. of water) 1 minute, washed, dried and mounted.

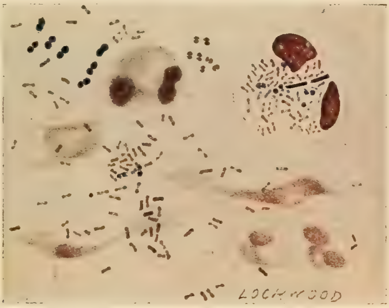

Fig. 14.—Sputum of influenza stained with Gram's and Bismarck brown, showing the Bacillus influenzæ (brown), Micrococcus pneumoniæ, *et al.* (blue). × 900.

Cultivation of Bacillus Influenzæ—Avery's Oleate-hemoglobin Medium.[32]—Preparation of the medium. Meat infusion agar (2%, see below) which is neutral or slightly alkaline in reaction is used as a base. To this is added a solution of sodium oleate sufficient to make a final concentration of 1 : 1000. A serum-free suspension of red blood-cells in broth is freshly prepared from sterile defibrinated rabbit's blood. One cubic centimeter of this corpuscular suspension is added to each 100 c.c. of oleate agar, the addition of blood being made while the medium is still hot. Plates are then poured, each containing about 15 c.c. of the oleate-hemoglobin agar and are used fresh to avoid the drying out of the medium. In the preparation of oleate-hemoglobin agar attention should be given to certain details:

1. *Agar.*—Two per cent. meat infusion agar having a reaction of from 0.3 to 0.5 acid to phenolphthalein should be used. The initial hydrogen ion concentration of the agar should represent a pH of from 7.3 to 7.5. Hormone agar prepared according to the formula of Huntoon [33] yields excellent results.

2. *Sodium Oleate.*—Two per cent. solution of sodium oleate (neutral) is made in distilled water, sterilized in the autoclave and kept as a stock solution. Five c.c. of this 2% solution of oleate is added to 95 c.c. of agar, giving a concentration of 1 : 1000.

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[32] Avery, Jour. A. M. A., 1918, vol. 71, p. 2050.

[33] Jour. Infect. Dis., 1918, 23, 169.

In the present work Kahlbaum's sodium oleate has been used, but other preparations are serviceable.

3. *Suspension of Red Blood-corpuscles.*—Sterile defibrinated rabbit's or human blood may be used. Since serum is known to inhibit the action of oleate and since hemoglobin is the constituent of blood essential for growth of Bacillus influenzæ, a serum-free suspension of the red corpuscles is used instead of whole blood. The red cells are removed from the defibrinated blood by centrifugalization, the supernatant serum is pipeted off and the corpuscles are made up to the original volume of blood by the addition of broth. One cubic centimeter of this suspension of red cells is added to each 100 c.c. of oleate agar. The suspension of blood-corpuscles should be added directly while the medium is hot and just before the medium is to be used.

4. *Formula.*—This calls for:

Agar	94 c.c.
2% solution of sodium oleate	5 c.c.
Suspension of red blood-cells	1 c.c.

Oleate-hemoglobin bouillon may be prepared in the same way by substituting broth in the place of agar in the foregoing formula.

Cultures taken from the nasopharynx by the West tube or from the throat by direct swabbing should be streaked on the surface of the oleate-hemoglobin medium according to the technic described for the detection of meningococcus carriers.[34] Similar plates should also be made from the sputum. At necropsy, cultures should be taken from scrapings of the tracheal and bronchial mucosa, as well as from the lung exudate.

All culture plates should be incubated for 48 hours at 37° C.

Pfeiffer's bacillus is an ubiquitous organism. To explain this its friends maintain that it has persisted with diminished virulence in areas formerly visited by epidemic influenza. Lord found it in 60 of 100 cases of non-tuberculous pulmonary infections with cough and in 29 of these in pure culture. This has been confirmed by many others.[35] It has been isolated from a surprisingly large number of cases of chronic bronchitis, asthma, tuberculosis, etc. The duration of these cases had varied from months to years, and one probably dated back for forty-five years.

It occurs in bronchopneumonia as a primary and as a secondary (*e.g.*, in diphtheria) invader. It is reported as a very common secondary invader in pertussis, after the paroxysmal stage begins (Bordet's bacillus?), in the bronchitis of measles, in lobar pneumonia and in tuberculosis with cavity formation. But what is more important it has been reported as the primary cause of acute bronchiectasis, of cholecystitis, arthritis, pyelitis, cystitis, otitis media, acute nasal infections, empyema, meningitis, endocarditis and of general septicemia.

Not only is Bacillus influenzæ the cause of conditions which in no way resemble epidemic influenza, but even the most typical of grippe epidemics have been shown to be due to quite other organisms than this; to Micrococcus pneumoniæ, Streptococcus pyogenes (hemolyticus and viridans), Streptococcus mucosus, etc.[36] Of a group of 31 cases of measles which developed a few weeks after an epidemic of influenza this bacillus was found in the sputum of 25.

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[34] Standard Technic of Meningococcus Carrier Detection, Adopted by the Medical Departments of the United States Army and Navy, 1918.

[35] Holt, Arch. Int. Med., 1910, v, 449; Leutscher, Arch. Int. Med., 1915, xvi, 657; *et al.* For very complete bibliography see Arnold, The Jour. of Lab. and Clin. Med., July, 1920, v, 652.

The opportunities afforded in 1918–20 to study the relation of this organism to the pandemic influenza were improved by many of the best bacteriologists of the world and their results have on the whole demoted this organism to a subordinate position. It certainly does not cause the primary infection called influenza or "flu"; it is one of the earlier secondary invaders, an important member of the second of the 3 or 4 "crops" which follow each other; while it may cause some but not all of its complications it would seem to have a definite place in the series of infections preparing the way for others, the various streptococci and pneumococci. So far as the influenza pneumonia is concerned the reports from different camps varied much, showing marked regional differences in the disease.[37] During an epidemic in Nashville, Tenn., 1920, Arnold[38] found Bacillus influenzæ in 35% of normal throats, in 77.7% of the cases of acute rhinitis and pharyngitis and in 86.5% of the cases of influenza.

Bordet's bacillus, the generally accepted cause of pertussis, is found early in pure culture in the tough masses of mucus and especially in the shreds of mucus from the smallest bronchi. It is decolorized by Gram's method and shows marked polar staining. Morphologically it resembles Bacillus influenzæ, although as seen in the sputum it is rather longer and plumper than is this "but as a result of cultivation it becomes smaller and smaller until it frequently appears as a mere point under the highest powers of magnification" (Bordet). This organism does not grow on ordinary media, but on media which are weakly acid and poor in nutrient constituents and which contain ascitic fluid or blood. The first growth is seen in two or three days. It can be trained to grow on media which do not contain blood. Bordet advises the following medium: 100 gms. of potato are cut into small slices and mixed with 200 c.c. of water containing 4% of glycerin and then heated in an autoclave. The fluid is then decanted. To 50 c.c. of this extract of potato are added 150 c.c. of a 0.6% solution of sodium chloride and 5 gms. of agar-agar. This is then autoclaved. While warm it is filtered into test-tubes, 2 or 3 cm. into each tube, and sterilized. Blood (preferably human, although guinea-pig's blood will do), is defibrinated and added to the agar of each tube in equal quantities. The tubes are then shaken and slanted.

Bacillus diphtheriæ or the Klebs-Löffler bacillus (Fig. 15), is a small straight or slightly curved rod, from 1 to 6, average 2 to 3, microns in length. It is a non-motile, non-liquefying, non-spore-producing aërobe.

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[36] Davis, Arch. of Int. Med., vol. xi, No. 2, p. 124.
[37] See Opie, *et al.*, Jour. A. M. A., 1919, lxxii, 108.
[38] *Loc. cit.*

It grows on all ordinary culture media, providing they are not acid nor too alkaline, but best on blood-serum.

One of the best media for this organism is *Löffler's blood-serum.* This is a mixture of blood-serum, three parts, and bouillon containing 1% of glucose, 2 parts. It is coagulated at about 70° C. When no tubes of media are at hand, cultures can be made on the coagulated white of a hard-boiled egg. The shell is lifted at one end of the egg, the surface inoculated and the shell put back. The egg is then put in a thermostat.

Bacillus diphtheriæ grows with great rapidity on the above-mentioned serum. At the end of even 8 hours in some cases the growth can be seen and the organisms easily found in smears, but a negative examination at that time has no value. In any case the growth can be determined in 18 or 20 hours. Until this time the diphtheria bacillus if present will have dominated and smears from the surface of the serum will look like pure cultures. From this time on, however, the ordinary organisms from the throat begin to dominate and will soon crowd out the diphtheria bacillus, unless the latter happened to be present in pure culture. The colonies of Bacillus diphtheriæ on blood-serum are moderate in size, elevated, of a grayish-white color and with opaque centers.

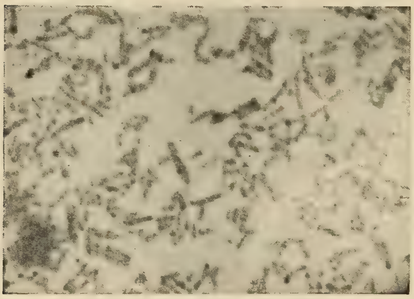

FIG. 15.—Bacillus diphtheriæ, from a young blood-serum culture. Photomicrograph by Dr. Thomas M. Wright.

This organism, if taken directly from the throat or from a young culture (less than 24 hours old), presents when properly stained an almost characteristic appearance. Smear preparations for microscopic examination are made by scraping some of the growth from the surface of the serum with a platinum needle. This is then rubbed on a clear glass slide, allowed to dry in the air and the slide passed through the flame 3 times in order to fix the specimen.

A good stain for this organism is *Löffler's Methylene Blue* (saturated alcoholic solution of methylene blue 30 c.c., and aqueous solution of 1 : 10,000 KOH 100 c.c.). The slide, covered with this stain, is warmed for a few minutes (it will not overstain), the stain then washed off in running water and the smear dried with blotting paper. The specimen can be improved by washing it further with 0.1% acetic acid, but this is seldom necessary.

Neisser's stain, intended to differentiate this organism from all others, has been adopted by many as the standard stain. The specimen is stained for about 5 minutes with a methylene blue solution (methylene blue, Grübler, 1 gm., 96% alcohol 20 c.c., distilled water 950 c.c., glacial acetic acid 50 c.c; this stain is filtered before using). During the staining the dye should be frequently renewed and the specimen gently heated. The stain is then washed off with water. It is next stained in Bismarck brown solution for 2 minutes (Bismarck brown 2 gms. dissolved in 1000 c.c. of distilled water). The polar granules are stained a deep blue and the bodies of the bacilli a light brown color.

Bacillus diphtheriæ is not decolorized by Gram's method, that is, it is a "Gram positive" organism.

Gram's Stain.—The smear is stained for 1½ minutes with aniline-gentian-violet (saturated alcoholic solution of gentian violet 5 c.c., aniline water 100 c.c.; the aniline water is made by slowly mixing aniline oil, 1 part, with distilled water, 20 parts; the mixture is allowed to stand for some hours and then filtered until clear). It is next washed in water and then put for 1 or 2 minutes in Gram's or Lugol's iodine solution (iodine 1 part, potassium iodide 2 parts, water 300 parts). The specimen is then washed in absolute alcohol for from 3 to 5 minutes. It may now be mounted, or it may be counterstained with Bismarck brown, washed, dried, and mounted. Bacilli which "stain by Gram," or are "Gram positive" retain the gentian-violet color. Those which "decolorize by Gram" or are "Gram negative" will be unstained unless a counter-stain is used.

Diphtheria bacilli stained by the above methods show at their ends or along their bodies deeply staining blue granules called "polar granules." Some bacilli contain so many such granules that they have a beaded appearance. This irregularity in staining as shown by the polar granules or the beaded appearance depends in part on the age of the growth and may entirely fail. These bacilli vary also much in size. Some recognize a "long" form and a "short" form and think these forms differ in virulence. But no relation between length and virulence has been determined. The length of the bacilli seems to depend rather on the stage of the disease when the culture was made and on the age of the growth.

For routine laboratory examinations, smears are made and blood-serum tubes are inoculated at the same time from material obtained directly from the throat or from the swab used. Smears are made from the culture before the growth is 24 hours old. If typical appearing bacilli are found in either of these two sets of smears, both stained by Neisser's method, a positive diagnosis is made. The smears made directly from the throat may show diphtheria bacilli and no growth be obtained, and frequently the growth will succeed when the search over the first smears was negative.

One searches the smears for bacilli about 5μ long with brown bodies and 2 blue staining polar granules, 1 at each end. If any answering this description are found a positive diagnosis of diphtheria is made. Hosts of other shapes and forms may be seen, but the presence of this form is considered conclusive. The barred and beaded forms are not as common as these with the two polar granules which are present in over 90% of the cases. If the culture is over 24 hours old when examined these forms may not be seen and such a culture should be discarded. One may then find a host of involution forms with their bizarre shapes: spindle, pear, dumb-bell, lancet, club, and the varicosed forms. These may be found in smears made directly from the throat. They cannot be differentiated from the involution forms of other mouth organisms.

If the organism were to be cultivated for several generations, and for diagnostic purposes this is never done, it would rapidly lose its characteristic morphology and could not be accurately differentiated by its morphology alone from other bacilli. For these reasons the routine described above is accurately followed in making clinical laboratory examinations for diphtheria and if typical forms are not found a fresh bacteriological examination is made. The statement is often made that Bacillus diphtheriæ can be found in the throats of healthy persons who have not been exposed to diphtheria. McCollum states that Löffler found it in 4 of 160 such individuals, Park and Beebe in 8 of 330, Kober in 5 of 600 and Denny in 1 of 235, but that he could not find it in any of the 130 such persons he examined. McCollum doubts also that it is often found in the throats of healthy persons who have merely been exposed to this disease, since he failed to find it in the throat of a single one of 60 nurses from the diphtheria wards of the Boston City Hospital. Some of the earlier reports of the presence of diphtheria bacilli in the throats of normal individuals may have been made before the question of pseudo-diphtheria bacilli had arisen, but it is also possible that some of these so-called normal persons had had a mild diphtheria, for some diphtheria patients do not realize that they are ill, that is, that they have a definite inflammation due to this bacillus in their throat or nose. It is also true that Bacillus diphtheriæ can live for a long time in the throats and noses of those who have recovered from a latent attack of diphtheria.

The test of virulence is sometimes important for the recognition of the diphtheria bacillus. To make this test a guinea-pig is inoculated subcutaneously with a bouillon culture not over 48 hours old of the organism to be tested. The virulence of Bacillus diphtheriæ tends to diminish after 2 days of growth. The amount of the bouillon culture medium injected should equal 1% of the animal's weight. If the organism injected is Bacillus diphtheriæ, the animal will soon show symptoms of acute or of chronic infection. If acute, the animal will die in from 1 to 6 days, and at the seat of inoculation will be found extensive necrosis with a marked inflammatory reaction. There will be extensive edema of the abdominal wall, effusions into the serous cavities, hemorrhages into the adrenals, swelling of and hemorrhages into the lymph-glands and focal necrosis in various other organs.

If a chronic infection results, the animal will show paralysis similar to that in man, and will die in about 6 weeks.

Formerly all organisms with the morphology described above were considered Bacillus diphtheriæ. Since then various pseudo-diphtheria bacilli have been described, and the relation of these to Bacillus diphtheriæ is still a mooted question in bacteriology.

The following groups of organisms may be mentioned:

1. A bacillus whose morphology is typical, with typical cultural characteristics, especially the ability to form acid from glucose, and which produces the typical lesions in animals, is, in the opinion of all observers, Bacillus diphtheriæ.

2. Bacilli with typical morphology and typical cultural reactions, especially the ability to form acid from glucose, but which are not pathogenic to animals, may be called avirulent diphtheria bacilli. Roux maintains, however, that the ability of the organism to ferment the sugars is not an essential characteristic of the species.

3. Bacilli whose morphology is typical but which do not conform in their cultural reaction with the diphtheria bacillus and which are either nonpathogenic to animals, or do not produce typical lesions, may properly be called pseudo-diphtheria bacilli.

4. Finally, there are a number of organisms which resemble Bacillus diphtheriæ in many ways, but whose morphology is not the same.

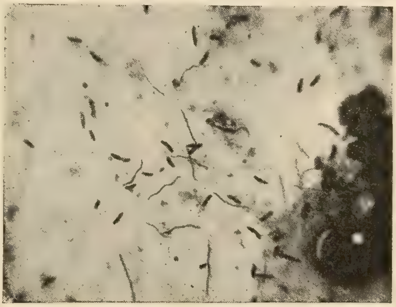

FIG. 16.—Smear from the throat of Dr. Louis P. Hamburger's case of Vincent's angina. Photomicrograph by Dr. H. Schapiro.

Bacillus Fusiformis and *Vincent's Spirocheta* (Fig. 16).—Bacillus fusiformis (Vincent) is a long slender bacillus often fusiform in shape, indeed the ends may be quite pointed. This bacillus is straight as a rule, but many are curved and a few may be S-shaped. It is non-motile (disputed), it decolorizes by Gram (disputed), is often beaded, and can be grown in pure culture.[39] It is best stained by carbolfuchsin.

Carbolfuchsin.—This stain is made up of basic fuchsin 1 part, absolute alcohol 10 parts, 5% carbolic acid 100 parts. The specimen may be covered with this concentrated stain for about half a minute, or, better, this stain is diluted with from 5 to 10 times its volume of water and then left on the smear for about 5 minutes.

This organism has been found in many normal mouths, on the surface of normal tonsils, in the cavities of decayed teeth, in the exudate of pyorrhea alveolaris, in antrum disease, aphthous ulcers, and also in fetid abscesses elsewhere in the body. This organism is common enough but has escaped notice since it is cultivated with difficulty and when seen in smears is usually passed by as a "harmless saprophyte." Recent work, however, is rather in favor of the view that this organism is pathogenic and pyogenic.

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[39] Weaver and Tunnicliff, Jour. of Infect. Dis., 1905, ii, p. 446.

In the mouth this organism is often, but not always, associated with a spirillum or spirocheta, also called the *Spirocheta of Vincent*, which measures from 15 to 25μ in length, is twisted in from 2 to 5 spiral turns, is actively motile, is Gram negative and stains so faintly that it is often overlooked. It has not yet been cultivated. This is quite certainly a saprophyte which occurs in enormous numbers in the mouths of even healthy persons and yet it is so frequently associated with Bacillus fusiformis that the two are supposed to be symbiotic and together cause the ulcers of Vincent's angina.

Sinclair (see page 59) believes that these organisms are important in explaining some of the hemorrhages of incipient tuberculosis. He examined the sputum for these organisms fresh and also cultured in broth under albolene and claims to get in 72 to 96 hours a growth of these dual forms.

Micrococcus tetragenus is a micrococcus which occurs always in tetrads, the individuals a little larger than the ordinary staphylococcus, their adjacent surfaces flattened and the group surrounded by a thick mucous capsule. Occurring usually in the mouth as a saprophyte it is, however, sometimes pathogenic and is found in the sputum in cases of bronchitis, tuberculosis with cavity formation and in hemorrhagic infarction. It is supposed to aid Bacillus tuberculosis in its destructive processes. Some would separate a pathogenic variety, which can be cultivated, from the common, harmless mouth saprophyte which will not grow on laboratory media.

Sarcinæ are seldom found in the sputum. One does occasionally find them in cases of gangrene, tuberculosis, bronchitis (see page 72), pneumonia, and in the sputum of old, debilitated persons in whom they often are the cause of the gray patches of stomato-pharyngomycosis sarcinia. They probably are harmless saprophytes.

PATHOGENIC YEASTS are much more important causes of pulmonary lesions than is generally believed and may explain many of the cases of chronic lung trouble under treatment for tuberculosis. Busse was the first to call attention to these organisms. In his case of "saccharomycosis hominis" due to *Saccharomyces busse* the original infection was of the tibia, but later there developed in both lungs caseous cavities in which the yeast was present. The yeast cells were rather small, averaging about 8μ in diameter, oval, very refractive and resembled fat droplets except for their greenish shimmer. The younger cells were homogeneous, but the protoplasm of the older ones is granular and the nucleus visible. They are made clearer by the addition of sodium hydroxide. Dr. Breed [40] states that after her attention had been directed to the presence of these organisms in the sputum of cases supposedly tuberculous she made cultures of all sputa in which on careful examination no tubercle bacilli had been demonstrated and in less than 2 years found at least 10 cases of possible pulmonary saccharomycosis. Of course yeasts are not rare as secondary invaders in tuberculosis. To find them the sputum is washed in tenth-normal sodium or potassium hydrate and cultures made from the particles resembling pus. Dr. Breed grew these yeasts on practically all the common media and tested the pathogenicity of the organism to rabbits, white mice, guinea-pigs and monkeys.

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[40] The Arch. of Int. Med., August 15, 1912, vol. x, No. 2, p. 108.

A patient was admitted to the Indiana University Hospital with many sinuses of several months' duration on the upper anterior chest wall, pus discharging. Some of these certainly led to the deeper tissues including the costal cartilages. Almost pure cultures of a slowly growing yeast were obtained from the sputum as well as from pus aspirated from the deeper portions of the sinuses.

BLASTOMYCOSIS.[41]—Blastomycetes (Fig. 17) are budding protophytes belonging to the same group as yeasts (Fig. 71, page 363). These organisms, round or oval in shape and from 8 to 12μ in diameter, have a finely granular often vacuolated protoplasm and a capsule with double contour separated from the protoplasm by a clear zone, often wider on one side than on the other. Reproduction in the living tissue is by budding only; in cultures, by mycelium formation. These organisms are pathogenic to rabbits, producing general systemic infection if injected intraperitoneally or intravenously. Subcutaneous inoculations are usually unsuccessful. They grow well on glycerin, glucose agar, blood-serum, bouillon, and other ordinary media. The growth at room temperature is microscopically visible in from 2 to 14 days, is dry, and develops many aërial hyphæ; that in the incubator is pasty and moist, with fewer aërial branches.

Thus far a few cases of general blastomycosis in man have been reported, the majority among foreign-born patients in Chicago. The symptoms suggest tuberculosis and the correct diagnosis has seldom been made before the subcutaneous abscesses had appeared. In practically every case the lung is involved sooner or later; indeed, evidence would indicate that in at least 65% of all cases the primary infection is in the lung. The organism may be demonstrated in the blood, urine and sputum. In the sputum one finds these cells in enormous numbers. It is probable that they are present in the sputum very early and yet unfortunately the diagnosis has not yet been made from sputum examination. The general character of the sputum will depend on the pulmonary lesion, whether there is present bronchitis, bronchopneumonia or frank pneumonia, small metastatic abscesses, cavity formation or fibroid changes with or without dilatation of the bronchi. While the characteristic sputum is abundant and very blood-stained, the patients may for long periods expectorate only clear mucus or a mucopurulent sputum.

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[41] See Montgomery and Ormsby, Arch. Int. Med., Aug., '08, vol. ii, No. 1, p. 1; also Fontaine, Haase and Mitchell, Arch. Int. Med., Aug., '09, vol. iv, No. 2, p. 101.

C. K., No. 9391, aged 59, was admitted to the surgical ward March 25, 1920, for multiple abscesses especially of the extremities, due to blastomycosis. He died April 10, 1920.

His sputum was a clear, white, very ropy mucus which contained very many of these parasites (see Fig. 17) and no red blood-cells. At autopsy both lungs were found to be the seat of extensive bronchopneumonia which showed no evidence of secondary pyogenic infection.

On March 27, 1920, the red blood-cell count was 4,000,000 and the hemoglobin 75%. The leucocyte count was 10,200, of which 8.3% were small mononuclears, 0.8% large mononuclears, 90.3% polymorphonuclear finely granulars and 0.5% eosinophiles. The morning urine had a specific gravity of 1.020 and 1.022, was straw-colored, turbid, contained a trace of albumin, no sugar, no casts, but very many yeast cells.

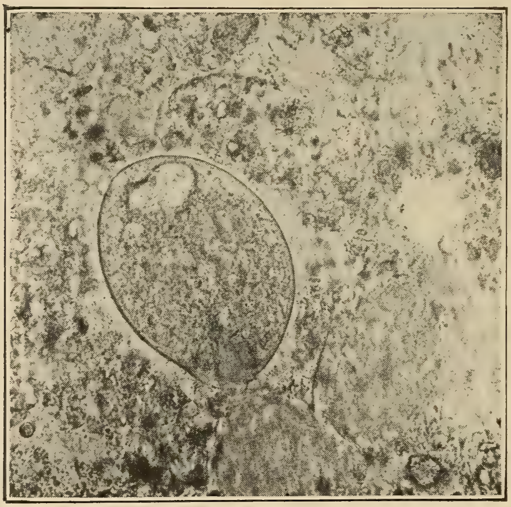

FIG. 17.—Balantidium Coli photographed in Stool (after MacCarty; Barker's Clinical Diagnosis, D. Appleton & Co.)

COCCIDIOSIS—COCCIDIOIDAL GRANULOMA—CALIFORNIA DISEASE.—Thus far but about 40 cases of infection with *Oidium coccidioides* or *Coccidioides immitis* have been reported, nearly all from the San Joaquin Valley, California.[42] This organism is not to be confused with blastomycetes and the clinical picture of this infection is different from blastomycosis.

Oidium coccidioides, Coccidioides immitis, belongs to the same group as the yeasts and the blastomycetes. It differs from the latter in that, while the blastomycetes multiply in the living tissues by budding only, this multiplies solely by endosporulation.

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[42] Hektoen, Jour. A. M. A., Sept. 28, 1907, vol. 49, p. 1071. In this paper 17 cases are reviewed.

Most patients with this infection belong clinically to the pseudotuberculosis group. "In fact, this disease presents the best mimicry of tuberculosis ever seen" (Hektoen). In most cases the initial lesion would seem to be in the lungs, which at autopsy are studded with disseminated miliary tubercles, areas of bronchopneumonia, or pulmonary abscesses. The sputum is mucopurulent or blood-streaked and in several of the cases contained the organism.

Oidium albicans is the very common parasite of thrush. This membrane can develop in the bronchi as well as in the mouth and pharynx. One sees it most often in the mouths of children, especially the weak babies, during their first week and of adults weakened by old age or disease, especially by diabetes or typhoid fever. In these cases we may find this growth in the throat, nose, esophagus, bronchi and lungs. The most common form found is the large-spored variety which liquefies gelatin in culture rather than the variety which produces smaller spores and which does not liquefy gelatin. In the sputum may be found these cells which resemble ordinary yeast in all particulars. They are from 5 to 6μ long and 4μ wide, oval in shape. When subjected to unfavorable cultural conditions this organism may grow in threads of very variable size and length, with double contour, the protoplasm of which contains droplets, granules and vacuoles. In these threads develop true endogenous spores.

Actinomycosis infection (*Actinomycosis bovis*) of man is rare in America. In about 15% of the cases reported the disease would seem to have been primary in the lung. Clinically, the picture suggests tuberculosis, while the sputum presents no distinctive feature other than the actinomyces granules. Some cases resemble a miliary tuberculosis; some begin as a slight catarrhal bronchitis which soon develops into a subacute bronchitis, with mucopurulent sputum often blood-streaked. In most of the cases, however, a bronchopneumonia is present from the first. These consolidated areas break down forming cavities which contain fluid, pus, fatty detritus, fat globules, degenerated red blood-cells and the sulphur granules. The ulcerating process progresses steadily through the lung and pleura to the chest wall which it perforates. This disease is slow in its course and progresses without periods of improvement until death. The sputum may at first be scanty and odorless, consisting of pure mucus, but in most cases it is mucopurulent or purulent and often (in about one-half the cases) hemorrhagic and fetid. It is sometimes as rusty as in pneumonia, sometimes resembles current jelly, while few cases have terminated in fatal hemorrhage. Some patients have expectorated at one time a large amount of offensive yellow material which in at least one case gave the patient the sensation as if the mouth were full of sand which grated between the teeth from the presence of the granules (Lord). If the sputum be carefully watched a correct and early diagnosis can usually be made since the sulphur granules which one finds are characteristic of this disease. They are small granules varying in size, some requiring the microscope for their demonstration, some even 2 mm. in diameter. They are round and have a yellowish, grayish, greenish, or brownish color. Sometimes one finds many in the sputum, sometimes few. Microscopically they consist of a network of fine, twisted threads radiating from a center and at the ends of which are the characteristic club-shaped swellings, which when present in large number form a ring around the granular central mass giving it a radiating or star-like appearance.

It is a good rule in any atypical case of lung disease to exclude actinomycosis. One must, however, not mistake masses of mouth leptothrix and masses of degenerating cells for this organism.

Moulds are often found in sputum, since special precautions in collecting and handling the sputum would be necessary to avoid this common air and dust contamination. Eliminating these accidental contaminations, one finds moulds in the sputum of those cases only who have destructive processes of the lung. Whether these "bronchopneumomycosis" are primary or secondary has been a much disputed point. Virchow believed that mould infections were usually secondary but that they could be the primary infection in necrotic tissue, for example in areas of hemorrhagic infarctions which they would transform into cavities. While one often finds a few moulds in tuberculous cavities, in others they are the prevailing organism. The contents of such cavities are as a rule odorless. There would indeed seem to be such an antagonism between moulds and the bacteria of decomposition that a cavity filled with the former is protected against the latter and vice versa. It is possible therefore that what would seem to be primary mould infections are those in which the moulds merely aided other organisms in the necrosing process and then crowded out the primary invader. Recently, however, through the work of the French, also of Saxer and others, it seems clear that Aspergillus fumigatus can be the primary invader and cause by necrosis an odorless cavity.

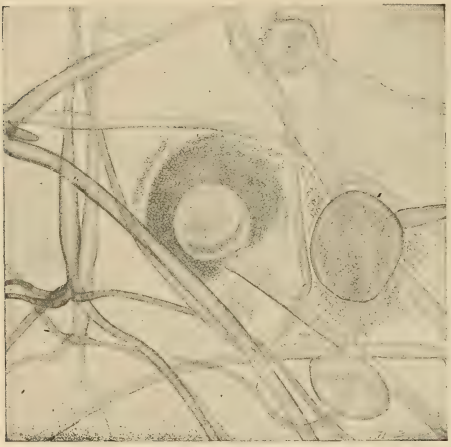

Fig. 18.—Mucor mucedo. × 60.

Among the pathogenic moulds are:

(1) *Mucor.*—There are 130 varieties of mucor, 6 of them definitely pathogenic. Mucor moulds are a very common air form. The group as a whole is characterized by the mycelial growth, which branches much and which at first is unicellular although later septa may develop, and by

its sporangia which develop at the end of erect hyphæ and consist of a columella surrounded by masses of spores enclosed by a membrane. Fig. 18 represents Mucor mucedo, a very common, harmless form. If a mucor mould be found in the sputum the observer should note carefully the shape of the columella, the size of the spores and the nature of the membrane, although for positive identification cultures are necessary. There are several varieties of mucor known to be pathogenic: Mucor corymbifer is a fine, delicate, small mould the spores of which are 2 by 3μ in size. Its sporangia are colorless and pear-shaped, varying in size from 10 to 70μ, and its membrane transparent. The columella, evident only when the spores have dropped off, is colorless and shaped like a boy's top, the larger end distal. This mould is in man the most common cause of kerato-, oto-, pharyngo-, and pneumomycosis. The sporangia-bearing hyphæ of Mucor rhizopodiformis are single, or branch as in a sheaf, short and of a brownish color. The sporangia are globular, black when ripe and the membrane opaque and soluble in water. The columella is brownish, from 50 to 75μ wide, is constricted at its base, which is also truncated and with a wide flat apophysis to the margin of which the membrane is attached. The spores are colorless, spherical and from 5 to 6μ in diameter. Mucor racemosus has spores from 5 to 8μ long and 4 to 5μ wide. Its columella is elliptical in shape. Mucor pulsillus has sporangia which are black, from 60 to 80μ wide and covered by a thorny membrane. The columellæ are egg-shaped or spherical, light brown in color and from 50 to 60μ wide. The spores are very small, round, colorless and from 3 to 3.5μ in diameter. Mucor septatus has a pale, grayish brown, spherical sporangium and small colorless columella which after the loss of the spores may grow still longer. The hyphæ have septa, hence the name of this mould. Its spores are about 2.5μ in diameter. Mucor racemosus has black sporangia which are 70μ in diameter. The membrane is transparent, the columella round, the spores colorless, opaque and from 3 to 4μ wide and 5 to 6μ long.

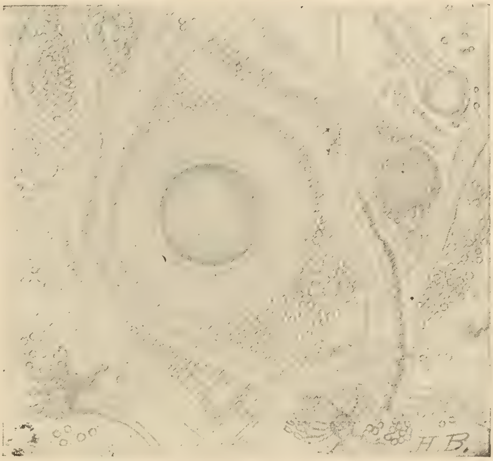

FIG. 19.—Aspergillus fumigatus. × 300.

The above forms of mucor are known to be pathogenic; almost all of them have been demonstrated in the ear. It is interesting that in all literature only 4 cases are cited in which they have been demonstrated in the lung and, so far as we know, in none of these cases were they found in the sputum before death.

Aspergillus Fumigatus (see Fig. 19).—This is by far the most important of the pathogenic moulds. Its mycelium is a thick mesh of threads from 3 to 6μ wide, the finest without, but the oldest with, septa. The conidia-bearing hyphæ are short, club-shaped and from 8 to 10μ in diameter at the larger (distal) end. The sterigmata, unbranched and from 6 to 15μ long, radiate from a central point, thus giving the head a fan-like appearance. The conidia spores, a chain of which tips the end of each of the sterigmata, are round, colorless, and from 2.5 to 3μ in diameter. The size of these spores is important since those of Aspergillus glaucus are from 7 to 8μ in diameter. All parts of this mould have a color which varies from brown to dark grayish green. The spores can be found almost anywhere in nature, as one may demonstrate by exposing a moist piece of bread to the air for only a few minutes and then placing it in the thermostat. *Aspergillus flavus* (see Fig. 20) has conidia-bearing hyphæ which are from 7 to 10μ thick. The young head has a yellowish or green color according to whether it is dry or wet and is brown when old. The conidia themselves are round, of a sulphur yellow color and from 5 to 7μ in diameter. *Aspergillus niger* is of a chocolate brown color and has conidia from 3.5 to 5μ in diameter. The growth of *Aspergillus subfuscus* is of an olive-green to a black color. It strongly resembles Aspergillus fumigatus, but is more pathogenic. Of all moulds Aspergillus fumigatus is the only one that has been shown to cause a primary lung infection. Sticker has collected from literature 20 cases in which no other disease of the lung was present. In 16 of these Aspergillus fumigatus was found, in 4 cases the mould was doubtful. One of these 4 cases, reported by Osler, was a woman who for 12 years had expectorated masses of mycelium the size of a bean, grayish, and of a downy consistency. An interesting case of primary chronic "membranous" bronchitis due to Aspergillus fumigatus was reported by Devilliers and Renon.[43] The patient was a grain sorter. Fragments of membrane composed of the mycelium of this mould (recognized from cultures) were expectorated monthly. These casts were from

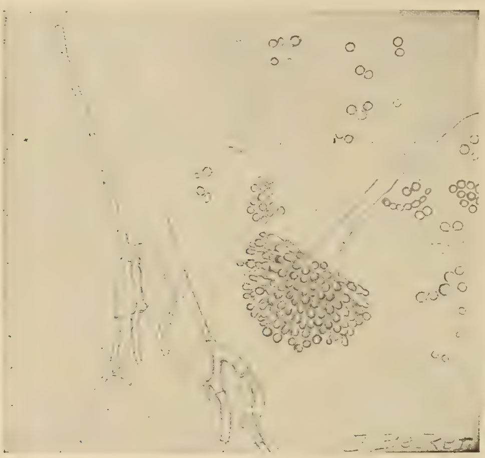

FIG. 20.—Aspergillus flavus. × 300.

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[43] Le Presse Med., 1899, ii, p. 325.

1 to 6 cm. long and, having no branches, probably originated in the larger bronchi.

Pneumomycosis Aspergillina.—Sticker [44] has divided the cases of aspergillosis into a "sporadic" group, which includes feeble patients susceptible because of their debilitated condition to such infection as well as persons suffering from other lung disease of which this is a secondary infection and an "endemic" group of cases who owe this disease to their occupation. Among the latter are the pigeon feeders, who are much exposed to the moulds of grain, and the hair combers, who work in an atmosphere so laden with infected dust that the cat is the only animal that can live with them. No autopsies on such cases have as yet been reported.

Clinically, many of these cases resemble chronic pulmonary tuberculosis and so form the *pseudo-tuberculosis group of mould cases.* The onset usually is with a hemorrhage, either slight or profuse, which recurs at intervals during the course of the disease. The cough at first is rather dry, then they raise a frothy sputum which often contains blood flecks. The sputum soon becomes greenish and purulent. Such sputum may be expectorated for months, even for years. Toward the end the expectoration is still greenish but more purulent, nummular, and sometimes more hemorrhagic.

In another group the process is a chronic bronchitis resulting in cirrhosis of the lung. The sputum of these patients is abundant, foamy, and watery. In Wheaton's case [45] the condition during life simulated actinomycosis, but at autopsy only a few tubercles were found and a large cavity. Some cases have expectorated casts of the bronchi made up of mycelium threads.

W., No. 1031, aged 47, admitted May 2, 1915, is a good illustration of this chronic bronchitic form of pulmonary aspergillosis. That these cases may give a distinctive clinical picture is suggested by the fact that this patient, admitted for pulmonary tuberculosis, when first met at ward rounds was at once demonstrated to the students as a probable case of pulmonary aspergillosis since the subjective symptoms and the physical signs were out of proportion to the effect the disease had had on the patient's general condition. The man had been considered tuberculous for many years, had been admitted twice to a special hospital for the tuberculous, although every one of the numerous examinations of the sputum had been reported negative. His dyspnea in the recumbent position was so great that for 9 years he had slept in a chair and yet during much of this time he had been able to earn his own living and had always maintained his weight. The widespread and marked physical signs of the lungs, the impaired resonance on percussion, the tubular modification of the breath sounds and the wide distribution of the râles which were moist and dry, coarse, medium and fine, suggested a very serious pulmonary condition and yet the patient had little or no fever and was able to live a fairly active life. The sputum contained the mycelial threads of Aspergillus fumigatus. For several years this patient returned periodically for the benefit of our classes in physical and clinical diagnosis.

While demonstrating him at ward rounds one day a visiting physician became much interested and said, "This must be what my wife has," which a later examination proved to be the case. (Mrs. K., aged 31, who had for years been considered a case of tuberculosis.)

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[44] Nothinagel's System, 1900, xiv.

[45] Trans. Path. Soc., London, vol. xliv, p. 38.

For the diagnosis of pulmonary aspergillosis the mould itself must be demonstrated in the sputum. In fact, the sputum should be examined for moulds and yeasts in all cases of suspected tuberculosis in which Bacillus tuberculosis cannot be demonstrated. One may find in the fresh sputum the mycelium threads, the conidia hyphæ, or the spores, yet the mycelial threads may not be found since they disintegrate rapidly. In dried and stained specimens one finds nothing suggesting a mould. It is important that the sputum of mould cases is usually odorless even though it contains large masses of lung tissue from a gangrenous lung.

Penicillium glaucum (see Fig. 21), the most common of our media contaminations, has segmented condia-bearing hyphæ which divide brush-like at the end, the branches being tipped by sterigmata which are flask-shaped. The conidia are from 2 to 3μ in diameter. This mould is non-pathogenic. Penicillium mummula certainly is pathogenic for animals and has been found in the ear of man.

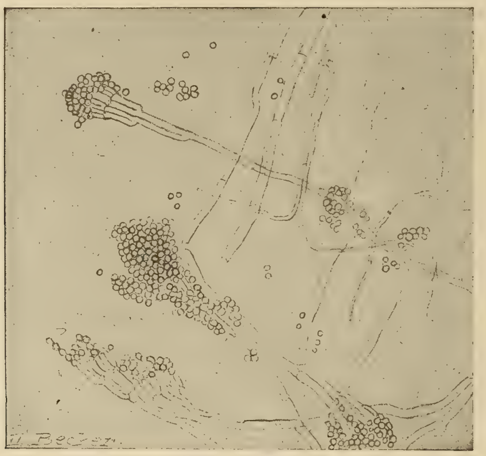

Fig. 21.—Penicillium glaucum. × 300.

Although one can recognize in the fresh specimen the general class of a mould if one can find a sporangium, *i.e.*, whether it is mucor, aspergillus, or pencillium, yet to determine the subvariety cultures are necessary. This may be done by spreading the sputum over a piece of bread as media, or by using Sabourand's medium (maltose, 3.7; peptone, 0.75; and water, 100).

The moulds may be stained in fresh specimen of sputum by a saturated watery solution of safranin or, better still, of thionin.

ANIMAL PARASITES

The **infusoria** found in the sputum are rarely important. Artault described *Ameba pulmonalis* as "a small ameboid cell which when stained looks exactly like a leucocyte, but while motile differs from it in its refractility." *Entameba histolytica* (see page 401) is found in the sputum of cases of liver abscesses which have perforated through the lung. *Endameba buccalis* is found in abundance in the pus of cases of pyorrhea alveolaris and so could of course be found in the sputum of such patients. This is probably the ameba found in the contents of abscesses of the jaw communicating with the mouth.

Flagellata have frequently been found in the sputum. A. Schmidt described such organisms in Dittrich's plugs; Artault, in the contents of a large tuberculous cavity; others in the sputum cases of lung gangrene and putrid bronchitis. In one case of abscess of the lung following pneumonia with operation 6 weeks after the onset of the pneumonia the sputum contained large numbers of flagellates. The name *Trichomonas pulmonalis* has been suggested for these protozoa and yet, so far as we know, they are identical with Trichomonas vaginalis (see page 404). Cercomonads (see page 404) have been found in the sputum and in Dittrich's plugs.

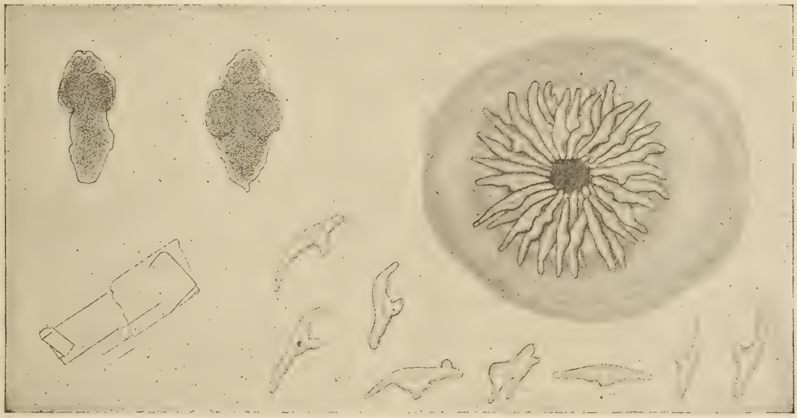

Fig. 22.—Sediment from echinococcus cyst. Above and to the left are two degenerated scolices (× about 60); to the right is the head of a scolex (× 400); below are hooklets of unusual shapes and a small mass of cholesterin crystals. × 400.

Echinococcus Disease.—Next to the liver the lung is the organ most often (in 16.8%) infected with *Tænia echinococcus* (see Fig. 22). If a pulmonary cyst bursts, or one in a neighboring organ (*e.g.*, the liver) ruptures through the lung, the sputum may contain daughter cysts, scolices, hooklets, or fragments of membrane from the cyst wall, any one of which is characteristic of the disease.

The echinococcus cyst wall (see Fig. 23) consists of 2 layers, an external laminated cuticular capsule and an internal granular parenchymatous endocyst. The cyst content is a clear, limpid fluid which has a specific gravity of from 1.005 to 1.015. It is neutral or slightly acid in reaction and contains considerable sodium chloride, but no coaguable albumin. Inosite, leucin, tyrosin, succinic acid and hematoidin have been demonstrated in this fluid. From the endocyst, buds develop which become smaller daughter-cysts and which break loose and lie free in the parent cyst. Inside these daughter cysts grand-daughter cysts may in turn develop. From the inner surface of the wall of any one of these cysts brood-capsules may form. These are cysts from the inner or outer wall of which the scolices grow. A scolex is the head of a Tænia echinococcus, and consists of a rostrum, which while alive it actively protrudes and retracts with 4 suckers and surrounded by a circle of hooklets. Sooner or later the parasite dies and the cyst wall degenerates, its contents become an inspissated mass of cheesy material containing many free hooklets and dead scolices which later may receive a coating of calcium carbonate.

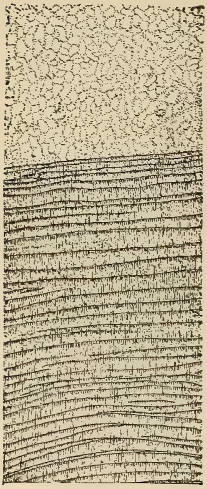

FIG. 23.—A small fragment of echinococcus cyst wall on cross fracture, showing transverse striation and pectination. × 50.

These cysts in the lung often lead to pulmonary gangrene; the cyst may rupture and its contents become infected, or the infection of its contents may precede its rupture. In either case the result is a cavity or cavities connected with bronchi.

The cough may at first be dry and hacking, and then as the cyst increases in size, some mucus may be expectorated. Hemoptysis is an early and important symptom. It may be the first indication of the disease. Preceding rupture there are merely blood-streaks or flecks in the sputum, but even then hemorrhage may be profuse. Rupture of the cyst is often accompanied by a profuse hemorrhage which may be fatal. When a cyst ruptures its cavity soon becomes infected and the sputum then is fetid pus, sometimes of a chocolate color resembling the contents of an hepatic abscess. If the suppuration of the cyst contents precedes its rupture the cyst then becomes a closed abscess cavity full of pus and the symptoms of its rupture will be those of rupture of an abscess. As a rule, unless some of the characteristic cyst contents is found in the sputum, cases of this disease are diagnosed phthisis or pulmonary gangrene. If daughter cysts and pieces of membrane are found in the sputum it means that the cyst has opened into a large bronchus. In these cases the sputum may for months contain pieces of this membrane.

PARAGONIMUS WESTERMANI.—This parasite, the "lung fluke," is the cause of the parasitical hemoptysis of man which is so common in Japan, parts of China, and Korea. In some mountain towns a majority of the people are said to be so infected. In Okayama 0.4% of all hospital cases admitted and in Kumamoto 5.9% of all pulmonary cases admitted showed this infection.[46] Stiles has reported 1 case in this country, that of a Japanese who had recently immigrated and who came under the care of Doctor Mackenzie of Portland, Oregon. A parasite which seems identical with this had previously been found in domestic animals in this country and it is perhaps only a matter of time before more cases will be reported from among our patients with "tuberculosis." The duration of the disease is long, usually from 10 to 20 years from the appearance of the first symptom. The sputum is generally scanty in amount, very viscid, and consists of small pellets of blood mixed with mucus. In some cases it is as rusty as that of pneumonia. When no blood is present the sputum still may have any shade of yellow or brown, or dirty red due to the eggs themselves when very numerous. Colored spirals resembling somewhat Curchmann's spirals are quite characteristic. The diagnosis of this disease rests wholly with the discovery of the characteristic eggs in the fresh sputum. These may be expectorated in large numbers. They have a thick, smooth shell of a dirty reddish color with a characteristic lid at one end, which is not always evident, or not exactly on the end, and which may be partly shelled off. These eggs (Fig. 24) are from 66 to 96μ long and from 48 to 60μ wide. The amount of blood in the sputum is usually small, at the onset but a few drops, but later even from 300 to 800 c.c. may be expectorated in a few hours, especially if the patient leads a laborious life. In some cases these severe hemorrhages recur with great frequency, in others the disease takes a very slow course. The hemorrhage is always arterial. Its cause is not clear because the ova are not contained in the pellets of the fresh blood. Charcot-Leyden crystals are common in this sputum, "sufficient proof that such crystals do not explain asthmatic paroxysms, since these cases never have asthma." [47]

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[46] Inouye, Zeits. f. klin. Med., 1903, vol. 1, p. 120.

Fig. 24.—Egg of Paragonimus westermani from the sputum of Dr. Mackenzie's case (through the kindness of Dr. Stiles). × 400.

Strongyloides Intestinalis.—Gage [48] reported a case of bronchopneumonia with larva of Strongyloides intestinalis in the sputum (see page 413).

Ransom,[49] suggests that the larvæ of *Ascaris lumbricoides* cause definite pulmonary symptoms when they pass through the lung from blood-stream to trachea and thence to the stomach and bowel.

CHEMICAL EXAMINATION OF THE SPUTUM

The chemical examination of the sputum offers but little of value in diagnosis and yet this little should not be disregarded. The determination oftenest made is that of the relative amounts of mucin and albumin. The test to be recommended is *Zeoni's modification of Schmidt's method*, a microchemical test. A small mass of the sputum is spread on a cover-glass, treated for at least a quarter of an hour with alcohol and then stained with a half-saturated aqueous solution of safranin (Schmidt used the Grübler-

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[47] See Stiles and Hassall, Sixteenth Report of the Bureau of Animal Industry, 1899.
[48] Arch. of Int. Med., April 15, 1911, vol. vii, p. 561.
[49] Jour. A. M. A., 1919, vol. 73, p. 1210.

Biondi stain). The specimen is then examined against a white background: the mucus stains yellow and albumin red. This method has the advantage over other somewhat similar tests that when much pus is present and therefore the albumin in the specimen as a whole considerable, one can estimate from the color of the background the relative amount of mucus and albumin in the serum of the sputum. In pneumonia the sputum contains much more albumin than in other conditions.

The soluble albumin may be estimated chemically by mixing the sputum with 3% acetic acid, shaking it well, allowing it to stand 12 hours, and filtering. The filtrate may be tested for albumin with potassium ferrocyanide, or it may be first neutralized, sodium chloride added, and the solution tested for albumin by the heat-acid test (see page 210). The amount of albumin may be estimated quantitatively in the Esbach tube (see page 216). After filtering out the precipitated albumin the albumoses may be precipitated by zinc sulphate or estimated from the amount of nitrogen present. Deutero-albumoses have been found but no peptone (Wanner). Wanner found by far the largest quantity of soluble albumin (0.3% to 0.6%) in the sputum of pneumonia; little, in that of bronchitis; and the merest trace, if any at all, in that of normal persons. The presence of albumin in the sputum means inflammation of the mucous membrane of the bronchi, not hypersecretion.

The quantity of mucin in the sputum may be determined by estimating the amount of glucosamin present, of which pure mucin contains 33.6%. To a weighed amount of sputum two volumes of alcohol are added, the fluid shaken, filtered through a hardened filter paper and the precipitate washed with alcohol. The precipitate is then boiled for 3 hours with 10% HCl in a flask with return cooler. The flask is then quickly cooled. Its contents is next made alkaline with NaOH, then acid with acetic acid. The biuret-giving bodies are then precipitated with phosphotungstic acid and the amount of reducing substance in the filtrate determined with Fehling's solution (see page 176). (Glucosamin has the same reducing power as glucose.) From 1 to 3.3% of mucin is present in the sputum of chronic bronchitis, a moderate amount in that of pneumonia (0.66 to 1.03%), from 0.74 to 0.79% in that of phthisis and none in that of bronchiectasis.

Wanner [50] considers that in the differential diagnosis between incipient tuberculosis and chronic bronchitis the presence of a definite trace of albumin in the sputum speaks in favor of the former; that the presence of considerable albumin indicates pneumonia or pulmonary edema; that in the differential diagnosis between pneumonia and infarction of the lung the presence of considerable albumin is in favor of the former. Goodman,[51] after very careful study, concludes that the quantitative estimation of albumin is not of very great diagnostic importance, since albumin may or may not be present in the sputum of pulmonary tuberculosis and is found frequently in the sputum of benign conditions. Its source is generally occult blood.

THE SPUTUM IN DISEASE

Pulmonary Tuberculosis.—"Pulmonary tuberculosis has no characteristic form of sputum" (Brown). "In the earliest stage of tuberculosis the sputum will be found negative for tubercle bacilli in from 60 to 75% of cases . . . therefore one or even half a dozen negative examinations mean nothing. Another very common error is that of assuming the case to be one of advanced tuberculosis because the symptoms and physical signs seem to clearly indicate it as such. Inasmuch as the diagnosis seems certain the sputum is not examined. It is because of this neglect that many cases of bronchiectasis, pneumokoniosis, chronic empyema, the mycotic infections and even chronic cardiorenal disease, are mistaken for tuberculosis" (Landis).

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[50] Deutsch. Arch. f. klin. Med., 1902, lxxv, 347; Fr. Muller, Ztschr. f. Biol., Bd. 52; here one finds the best discussion of mucin and its allied bodies in the sputum.

[51] Arch. of Int. Med., August 15, 1911, vol. viii, p. 163.

Cases of chronic fibroid pulmonary tuberculosis may have no, or very little (usually purulent), expectoration and the little sputum which some cases do raise may for a long time be free of tubercle bacilli.

In the pulmonic form of ACUTE MILIARY TUBERCULOSIS the patient has as a rule little cough and no sputum. When there is sputum it is mucopurulent and due to the acute diffuse bronchitis present. Patients with the acute bronchopneumonic type of pulmonary tuberculosis expectorate a rusty or blood-streaked sputum with an occasional hemoptysis. Tubercle bacilli if present probably come from an older pulmonary lesion. In acute pneumonic tuberculosis the sputum as a rule is identical with that of acute lobar pneumonia until the time when the crisis or lysis is due, then the appearance of green sputum may suggest the true condition. In acute pneumonic tuberculosis with extensive caseous consolidation there may for some time be no sputum whatever, but the majority of cases expectorate at first a mucoid, then a rusty sputum, which even as early as the fourth day may contain Bacillus tuberculosis. Later, in 8 or 10 days, the sputum becomes mucopurulent and greenish and then elastic tissue may be found.

Of 15 cases of acute tuberculous lobar pneumonia in the Johns Hopkins Hospital Clinic in 4 the sputum was typically rusty; in the majority it was a mixture of a rusty sputum with that of bronchitis; while 2 had practically no expectoration at all. The fresh blood was a marked feature of the sputum of nearly all these cases, while 2 had brisk hemorrhages. In the typically rusty sputum very few pus-cells were present, but many alveolar epithelial cells and intact red corpuscles. Its green color and tenacious consistency were marked in a few cases. After the first week the sputum became more mucopurulent and yet still somewhat blood-streaked, later nummular and in 2 cases positively foul. In 2 cases, however, the sputum was not at all bloody but mucopurulent from the first.

In those cases in which bronchitis precedes the tuberculous pneumonia there is an abrupt change in the sputum. It becomes reduced in amount, tenacious and blood-streaked. If a true acute lobar pneumonia develops in the course of chronic tuberculosis the sputum is that of lobar pneumonia mixed with that of bronchitis. In 1 case these did not mix but formed 2 layers in the cup, the upper mucopurulent and blood-tinged, the lower exceedingly tenacious. Later in this case and until death it was a very tenacious greenish pus. In another case elastic tissue was found in the sputum before the tubercle bacilli, although repeated examinations for the latter had been made. In one case, at first clinically acute lobar pneumonia, the sputum on the third day was markedly blood-tinged, contained tubercle bacilli and 2 bronchial casts about 1 mm. in diameter at their larger end. One patient with negative history so far as previous lung trouble is concerned had become ill suddenly 2 days before admission. For 9 days his sputum was white, sticky, mucopurulent. Then it became green in color and on the nineteenth day of his disease he suddenly began to expectorate abundant sputum which formed 2 layers, the upper sanguineous and the lower mucopurulent. On that same day tubercle bacilli for the first time were found.

In ACUTE TUBERCULOUS BRONCHOPNEUMONIA a hemorrhage is sometimes the first symptom. The sputum may from the first contain elastic tissue and tubercle bacilli.

In the extensive tuberculous bronchopneumonia which represents the terminal stage of this disease and especially in that form which follows an acute infectious disease, there may for weeks be no sputum at all while other cases will for weeks expectorate a sputum which contains no elastic tissue and no tubercle bacilli until necrosis and excavation begin.

E. N., No. 8288, 20 years old, was admitted June 26, 1919, with extensive bronchopneumonia, presumably tuberculous in character, which followed influenza. The temperature reached from 103° to 104° each afternoon. She had no cough and no sputum whatever till August 17 when she raised just a little which was negative on examination. The expectoration of the following day contained both elastic tissue and tubercle bacilli. She died September 18, 1919, just 30 days later. Autopsy showed extensive tuberculous pneumonia and multiple cavities.

M. G., No. 9612, aged 22 years, was admitted May 15, 1920, 7 months pregnant, with high fever and extensive bronchopneumonia with profuse expectoration. This was examined almost daily and was negative till June 19, 1920, when numerous bacilli suddenly appeared and were present each subsequent day until her return to her home soon after.

In CHRONIC ULCERATIVE TUBERCULOSIS the sputum may have almost any color and almost any character. It may be (Biermer) mucoid, mucopurulent, blood-stained, or almost pure blood. In amount it may vary from none to 1 liter in 24 hours and in consistency from that of glue to that of water.

In some cases, especially of early apex tuberculosis with marked physical signs and severe cough, there may be no sputum at all, but in the great majority of cases there is for weeks a slight morning sputum, although it may be necessary to urge the patient to expectorate this. In a few cases the onset is with a slight hemorrhage; in more, the sputum is at first mucus containing much myelin and hence is glairy and resembles boiled sago. There is nothing distinctive in the gross appearance of such sputum. This may be its character for months, but sooner or later one will find in it little caseous masses, the first sign of tuberculosis. Later in the course of the disease the sputum becomes more profuse and mucopurulent, resembling that of chronic bronchitis. As ulceration proceeds it becomes still more profuse, mucopurulent or purulent in character and yellow or greenish in color. Microscopically, it contains pus-cells, blood and epithelial cells of all kinds, including the alveolar epithelial cells filled with myelin, while if the sputum be hardened *en masse* and sections cut, giant cells may sometimes be found. The presence of Streptococcus hemolyticus in these cases would seem to be important.[52]

In the course of a case of chronic ulcerative tuberculosis various changes may occur. In cases of sudden heart failure there may be a cessation of sputum for 1 or 2 days without apparent injury. "A sudden disappearance of the sputum when before it had been abundant, especially in the morning, should always put us on our guard. Miliary tuberculosis is occasionally ushered in in this manner" (Brown). Again, the sudden appearance of an abundant mucoid, more or less frothy sputum may mark the onset of miliary tuberculosis (Brown).

For diagnosis it is most important to recognize in the sputum the small caseous particles, "rice bodies" (corpora oryzoides) since in them one has the best chance of finding tubercle bacilli and elastic tissue. To find them the sputum should be spread out on a dark plate or squeezed between the surface of 2 plates of glass (many prefer Petri's dishes which are more easily handled and sterilized) and then the whole surface should be scrutinized with a small hand-lens. They vary from about 0.5 to 1 mm. in diameter, are of a white opaque color, are more or less lens-shaped, have a bad odor, and when picked up with a needle and spread on a slide are found to be more brittle and claylike than are, *e.g.*, particles of food (see page 17).

Elastic Tissue (see page 14).—So important in the diagnosis and prognosis of cases of pulmonary tuberculosis is the presence of elastic tissue in the sputum that the search for it should be methodical and intelligent. One may look long and find none, while another choosing particles with care may find it easily in a few minutes. Excluding that from other sources (see page 17) its presence in the sputum always means necrosis of lung tissue. In tuberculosis one seldom finds the large fragments of tissue found in abscess of gangrene of the lung. On the contrary, the disintegration is molecular and the elastic tissue is expectorated in the very small, round particles mentioned above (also page 6), or in grayish threads, or even as single fibers (see page 14). These fibers come as a rule from the parenchyma of the lung and when present in clumps these may preserve the shape of the alveoli. Still other fibers come from the bronchi and the blood-vessels. In some cases elastic tissue is found early, before there is any suspicion of disintegration; in other cases, for instance, in caseous pneumonia, death may ensue before any is expectorated. In the majority of cases, however, its presence continues as long as ulceration continues and its amount in the sputum is a good index of the rapidity of this process.

Sputum from a Cavity.—Some of the older writers (as Winkel) have considered that the odor of the breath may help in the diagnosis of a cavity, since in certain cases the sputum in the cup is odorless, while the breath of the patient is most offensive (see page 5). The explanation would seem to be that warm sputum stagnating in a cavity may have a heavy, penetrating, sweetish odor which it imparts to the breath, but not so when cold.

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[52] Hayes, Am. Rev. of Tuberc., 1920, iv, p. 82.

During cavity excavation the sputum is mucopurulent and often is expelled in masses which flatten in the cup into coin-shaped clumps, the so-called "nummuli." The best examples of this nummular sputum are seen in cases of caseous pneumonia with cavity formation. The nummuli are green or dirty grayish green in color and consist chiefly of pus. They do not coalesce in the cup. They sink at once in water. Their odor is not always offensive. In them can be seen little dots, even as small as a millet seed, which consist of granular detritus, black pigment, elastic tissue and a few pus-cells. Masses similar to these nummuli may originate also in the larger bronchi in non-tuberculous chronic bronchitis.

When the softening is rapid the expectoration of 100 to 150 c.c. of sputum a day is not unusual. The sputum from large cavities is raised chiefly in the morning, and often contains blood. If the blood has been retained for some time in the cavity it becomes blackish in color. In case a tuberculous cavity communicates with a bronchus by a fine opening the sputum may form a skein similar to that seen in abscess of the lung (see page 78). Large fragments of lung tissue are rarely found in the sputum in tuberculosis and yet the connective tissue formation in the walls of a healing cavity may be rapid enough to dissect off large particles of the necrotic cavity wall which are expectorated.

While the sputum of cases of tuberculosis with cavity formation usually has a sickening, sweetish odor, should secondary infections (other than the secondary infections which lead to the cavity formation) lead to bronchiectasis, gangrene, or putrid bronchitis, the odor of the sputum may be foul; but it is remarkable how seldom this occurs.

As the cavity clears and becomes lined with connective tissue, the character of the sputum changes considerable. We then have the "sputum globosum," consisting of balls composed of a conglomerate of mucus, detritus and pus, of a grayish white color, thick, rounded, and shaggy, some of which, but not all, sink in water.

Hemorrhage.—Hemoptysis occurs at some time or other in from 60 to 80% of all cases of pulmonary tuberculosis. The amount of blood lost on one occasion varies from a drop to several cupfuls. Some patients have so many hemorrhages that they constitute the so-called "hemoptysical group." In one large group of cases the hemorrhages are early, sometimes the first symptom of this disease. These early so-called "inflammatory hemorrhages" are slight, apt to recur frequently and are similar to those seen in acute lobar pneumonia. Like these they are due to the escape of blood-cells by diapedesis or to superficial erosions of the mucosa. Later in the disease, however, the hemorrhages are of a very different character. They then are profuse and sometimes fatal, often occurring without warning in a person apparently recovered. These arise from the rupture of small miliary aneurisms of arteries which cross an old cavity or which are exposed in its wall.

Sinclair[53] believes that in Hawaii at least hemorrhage rarely occurs in incipient cases of pulmonary tuberculosis unless the Spirocheta of Vincent and Bacillus fusiformis are present; that when these are present hemorrhage occurs in 76% and when absent in but 36% of the cases. He admits that the presence of these organisms may be merely an indication of a mixed infection.

For the demonstration of and description of Bacillus tuberculosis see page 22.

One negative examination of sputum proves nothing. Some search for 3 days, others 6 or 7; we believe in searching as long as there is any sputum. Brown tells of a patient whose sputum contained tubercle bacilli only on the twenty-sixth daily test. "It is of doubtful value to put the sputum in the thermostat that the bacilli may grow."

The Prognostic Value of Sputum Examination.[54]—Sputum examination gives very little data of prognostic value. The reasons for this are the following: It is certain that not all of the tubercle bacilli in any given specimen will take the stain; the sputum from old foci may contain very few bacilli and that from young foci, however actively they may be forming, may contain none at all; by the occlusion of a bronchus the contents of an intense focus may not be expectorated for a time and when this bronchus does open, the sputum, formerly negative, may for a few days contain vast numbers of tubercle bacilli and then for months few or none; in some specimens the organisms are abundant in one portion and scanty in others; the sputum of some persons with fatal tuberculosis contain no tubercle bacilli at all (for instance, in cases of caseous pneumonia and acute miliary tuberculosis), while in other cases the bacilli are present even before one can elicit any physical signs of this disease; lastly, in severe cases of tuberculosis with acute bronchitis the bronchial secretion will so dilute the sputum that the bacilli may appear few in number. And yet the following points may assist us in prognosis:

The continued expectoration of large numbers of tubercle bacilli indicates the presence of a cavity; a sudden increase in the number of these bacilli, together with an increase in the number of cellular elements, suggests cavity formation; a steady decrease in the number of bacilli continuing over a considerable period of time would, if the physical signs agree, indicate improvement; a case should be called "healed" only after the bacilli have been long absent from the sputum. On the other hand, the continued presence in the sputum of large numbers of bacilli does not necessarily indicate the case is advancing. Fowler's patient, for example, for 14 years expectorated daily large numbers of tubercle bacilli and yet during this period the patient was in fair health and even improved. Trudeau has mentioned a similar case extending over a period of 10 years. Such patients, needless to say, are the source of the greatest danger to their neighbors expectorating as they may from 3 to 4 billion bacilli each day.

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[53] The Am. Rev. of Tuberc., 1920, vol. iv, p. 201.

[54] Brown, Montreal Med. Jour., October, 1901; Jour. Amer. Med. Assoc., February 21, 1903. The reader is referred to these articles, from which much of the above paragraph is quoted.

Many have considered the morphology of the bacilli more important in judging of prognosis than their numbers. They hold that while in all cases both long and short rods may be found, yet a predominance of short rods indicates rapid growth, that of long rods a slower growth. Brown believes that while in general the morphology of the bacilli gives but little aid, a predominance of short rods does indicate a rather active process. Others assert that the arrangement of the bacilli is important; that bacilli in clumps and parallel groups indicate a lively growth, and that groups of short bacilli mean a bad prognosis. Bacilli which stain deeply are considered to be especially virulent.

There certainly is truth in all of these beliefs, yet there are so many exceptions to each that they should not be taken too seriously.

The question is often asked, Is the discovery of 1 red bacillus in a properly stained sputum important? It would only be suggestive (see page 23). A positive report should always be confirmed. On the other hand, the failure to find bacilli in the sputum does not necessarily exclude tuberculosis.

A fairly accurate estimation of the number of bacilli in the sputum is possible [55] but for clinical purposes it is not worth the considerable trouble it involves.

Acute Lobar Pneumonia: Croupous Pneumonia.—Some patients at first and even during the entire course of a case of acute lobar pneumonia, raise no sputum. This is true of some old persons, some alcoholics, of infants and young children, very ill patients, and patients with diseases of which pneumonia is a complication. This is true often of the terminal pneumonias of chronic pulmonary tuberculosis, arteriosclerosis, heart and kidney disease, diabetes, etc. But there are also some strong adults, especially those with apex pneumonia, who raise no sputum during the course of the pneumonia. Some of these patients have more or less dry cough, while still others do not even cough during their disease, although the absence of sputum in a case otherwise suggesting acute lobar pneumonia always should arouse one's suspicions that the consolidation is not due to Micrococcus pneumoniæ.

At the onset of a case of acute lobar pneumonia the sputum is as a rule quite red, its color due to unchanged red blood-cells, and remarkably transparent, since these cells are not arranged in rouleaux but are scattered singly throughout the mucous mass. Some cases begin with a genuine pulmonary hemorrhage. Markedly hemorrhagic sputum, however, should arouse the suspicion of tuberculosis since it is very rare in acute lobar pneumonia unless the patients have also heart disease or the case is one of post traumatic pneumonia. In still other cases the sputum for even 4 or 5 days after the onset consists of white mucus, is abundant, and only later becomes hemorrhagic. One possible explanation for this is that the clear mucus is expectorated while the infection is limited to the bronchi, and that the appearance of blood indicates that it has reached the alveoli (Mueller). But whatever the sputum at onset, in from 1 to 3 days from the initial chill the great majority of patients with acute lobar pneumonia have the typical rusty sputum characteristic of this disease.

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[55] Nuttall, Johns Hopkins Hosp. Bull., May, 1891.

The sputum of few diseases is as distinctive when typical as this. In a doubtful case this alone may settle the diagnosis. It is yellowish brown in color, the color of iron rust, is homogeneous, glairy, almost transparent, and so tenacious and jelly-like that the cup often can be inverted without any spilling. Such was the sputum in 71% of our cases (counting the rusty sputum containing blood) and in 62% of Lord's cases. This rusty color is due to a pigment derived from hemoglobin and dissolved in the mucus; microscopically very few intact red blood-cells can be found. This typical sputum appears sometimes on the first day of the disease, more often on the second, but sometimes later, especially if much bronchitis also is present with its mucopurulent sputum. The sputum in pneumonia varies in amount from none to from about 150 to 300 c.c. per day. When small in amount it dries rapidly in the cup. Its tenacity, which is remarkable since it contains little mucin and much water, has been ascribed to nuclein in alkaline medium.

Mixed with the rusty sputum one usually finds fresh blood, often in dots or streaks of varying size, while in other cases the sputum for days is almost pure blood (see page 82). The extension of the pneumonia to another portion of the lung is often indicated by an increase in the amount of, or the reappearance of, fresh blood in the sputum.

While the characteristic sputum of acute lobar pneumonia is rusty in color, that of some cases has an orange-yellow, a lemon-yellow, or a grass-green color; in fact, all the possible shades which are seen in subcutaneous bruises. These colors are due to different oxidation products of hemoglobin (Traube). The sputum may appear jaundiced, but this term should never be used unless the skin is icteroid for one can practically always get a test for bile pigment in pneumonic sputum.

Microscopically, rusty sputum presents a transparent background in which are scattered a few red blood-cells, some intact but the most of them swollen and pale and not nearly numerous enough to explain the color; many epithelial cells, columnar or pavement; leucocytes; granular cells, and oil globules. Chemically this sputum is characterized by an absence of alkaline phosphates, an excess of potassium over sodium, an increased amount of sulphates, of calcium chloride and a large amount of soluble proteid.

Soon after the crisis the sputum may entirely cease, but more often it becomes more abundant, loses its rusty color, becomes mucopurulent, and finally is white mucus. "In no other (disease) is the cycle of sputum changes so marked or of so great diagnostic value as in this disease" (MacKenzie).

Of a series of 94 cases, 21% stated that they had no sputum at the onset of the disease; 46% had sputum but said that it was not bloody, whereas 33% stated that the first sputum noticed was slightly bloody. During the course of the disease 16% of the cases had little or almost no sputum; one case was in the hospital 17 days without any expectoration, and other cases about 7 days. In 32% the sputum was typically rusty; in 39% rusty and blood-streaked; in 3% very bloody; while in 10% at no time during the disease was any blood noted.

Variations.—If pneumonia develops in a case of chronic bronchitis (Traube) the patient usually expectorates a reddish mucopurulent sputum which is quite fluid and not at all rusty; in other cases it is a bloody, mucoid pus. In the "hemorrhagic pneumonia" of the aged the sputum instead of being rusty is very bloody. If pneumonia develops in a case of chronic passive pulmonary congestion due to heart, lung or renal disease, the patient expectorates a characteristic "brick-red" sputum which is as thin as that of pulmonary edema and very bloody. This is the so-called sputum of "congestion" or of "serous pneumonia" (Traube).

Green sputum in a case of pneumonia running an unusual course always should arouse suspicion. If the skin is jaundiced it has no significance and if associated with delayed crisis or lysis a perfect resolution may follow. But on the other hand it often indicates a serious outcome; it is sometimes the first symptom of abscess of the lung; and, lastly and most important, it may be the first hint that the diagnosis of croupous pneumonia was incorrect and that the case from the onset was one of tuberculous pneumonia. Such were Traube's cases with green sputum the report of which first called attention to this condition.

In cases of pneumonia which end in necrosis or gangrene of the lung, and this is true of 3% of the fatal cases of lobar pneumonia, the sputum presents a characteristic series of changes. It first loses its tenacious consistency and becomes more fluid, its color changes from "rusty" to "coffee," then to "prune-juice," and later to "chocolate" color. The red blood-cells disappear. The odor, at first absent, becomes stale and later decidedly fetid. Granular detritus appears and then fragments of necrotic lung tissue. In still others of these cases the sputum may be continuously "prune-juice" in character, but this is rare. This series of changes in the sputum may be sufficient for the diagnosis of pulmonary gangrene even before the necrotic fragments have appeared. The reason for these plays of colors is not clear since the red blood-cells in some cases have been described as well preserved. In about 4% of the fatal cases of croupous pneumonia abscess of the lung develops in the consolidated area (see page 79).

Prune-juice sputum in pneumonia has considerable clinical importance. It may indicate merely a severe type of the pneumonia; in still other cases it indicates an asthenic type; in others, and in these it has no sinister significance, it merely signifies the beginning of resolution; but it may, as mentioned above, warn us of the onset of pulmonary gangrene and, finally, its appearance replacing rusty sputum particularly in old persons may indicate a developing edema of the lungs.

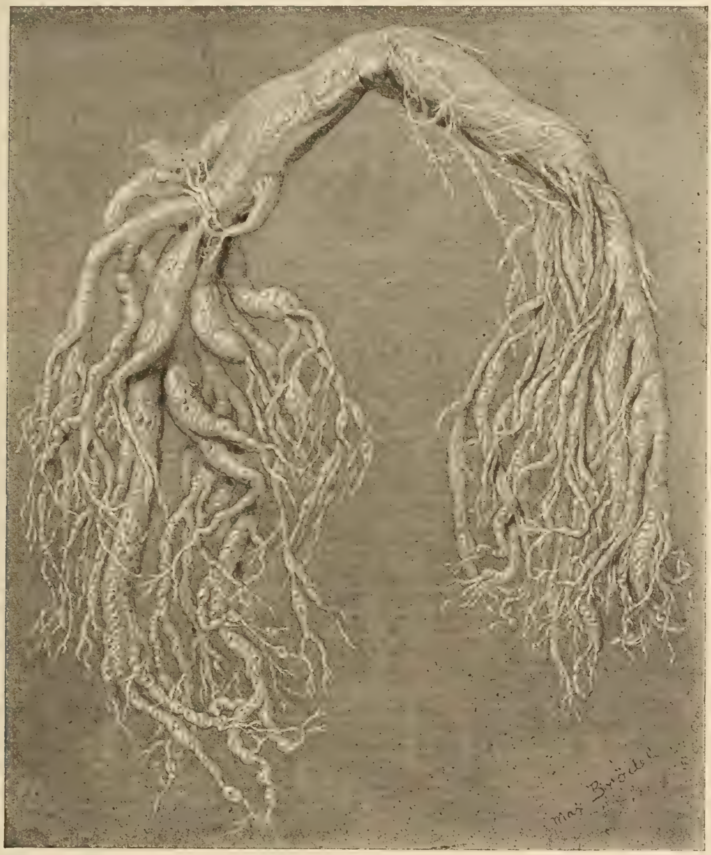

FIG. 25.—Fibrin cast from a case of double pneumonia. Natural size. The patient was a man 65 years of age. The cast was expectorated on the sixth day of a double pneumonia, followed by hemorrhage. Death on the seventh day.

Fibrin coagula (see page 9) are often present in the sputum of pneumonia, especially in cases of the massive type. They appear at first glance as unformed masses, but if shaken out in water are found to be beautiful branching fibrin casts of the bronchi, of varying size, the largest with hollow branches. Some have clots and small drops of blood in the lumen, especially at the bifurcations of the branches. One cast pictured in natural size as Fig. 25, seemed to be from 2 entire lobes. Curschmann spirals, and, in fact, every other constituent of the sputum of asthma, may be found in that of pneumonia. This was illustrated in Vierordt's case [56] of typical pneumonia but with intense general bronchitis. The sputum was bloody on the fourth and fifth days and contained many fibrin coagula. These were particularly beautiful on the seventh day, on which day spirals were also found. Resolution began on this day. From that time on were found beautiful Curschmann spirals but no Charcot-Leyden crystals.

MICROCOCCUS PNEUMONIÆ (see page 32) can as a rule be demonstrated in the sputum of cases of acute lobar pneumonia. Since, however, this group of organisms is found in the mouths of about 50% of normal persons (although its presence there is better determined by animal inoculations and by cultures than by smear preparations made from the sputum, see page 33), and since it is often found in small numbers in the sputum of patients suffering with various other lung diseases, its presence is not proof of a pneumococcus infection. On the other hand, its absence from the sputum of many cases of acute lobar pneumonia is not to be wondered at since at autopsy these organisms are found chiefly at the advancing edge of the involved area.

The term Micrococcus pneumoniæ is now used of a group of organisms and lobar pneumonia is no longer thought of as due to those of this group only. Cole found that 14 of the 237 cases he studied were due to the following organisms: Bacillus influenzæ (5), streptococcus (3), Streptococcus mucosus (1), staphylococcus (2), Bacillus of Friedländer (2) and one to a mixed infection of streptococcus, staphylococcus and influenza bacillus. Hastings and Boehm [57] found in the blood and in the sputum of many cases of typical lobar pneumonia organisms other than pneumococci; Streptococcus hemolysans and Streptococcus mucosus capsulatus were found on culture in the sputum and Micrococcus pneumoniæ in but 24 of the 44 cases of pneumonia they studied. The work of Cole and his co-workers (see page 33) is however the most important recent contribution to this subject.

LOBAR PNEUMONIA DUE TO FRIEDLÄNDER'S BACILLUS.—Friedländer's bacillus is found frequently in mixed infections of the respiratory tract but sometimes, in about 4% of the cases, would seem to be the cause of a lobar pneumonia, which at first was probably lobular; although even in these cases it is often considered a secondary invader. This pneumonia is followed by abscess formation and necrosis of lung parenchyma. It runs a very rapid fatal course.

In the cases of acute lobar pneumonia apparently due to this organism alone the sputum was very mucoid in character and blood-streaked, rusty, or truly hemorrhagic. Microscopically it contained many of these bacilli and but few cells. While one may find these organisms in the sputum in great numbers the only proof that they are the etiological agent would be a pure culture from material obtained by lung puncture, or from blood cultures.

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[56] Berl. klin. Wochenschr., July 16, 1883.

[57] Jour. Exp. Med., March 1, 1913, vol. xvii, p. 239.

The *pneumonia occurring in typhoid fever* is often of the true lobar type, especially that which occurs at the onset, the cases of "pneumotyphoid fever." The sputum of these patients is sometimes typically rusty and may contain Bacillus typhosus. A lobar pneumonia may develop during the course of typhoid fever (although bronchopneumonia is the more common form). These patients may raise no sputum whatever. It may develop also during convalescence.

In the *subacute indurative pneumonia* the sputum is usually abundant and may contain blood but is seldom rusty. There is a decided tendency for it later to become fetid. In chronic interstitial pneumonia the cough is often paroxysmal and the expectoration is as a rule copious, of a mucopurulent, or a seropurulent nature and sometimes fetid. Hemorrhage occurs in about one-half of the cases.

These forms of *chronic pneumonia* are often complicated by bronchiectasis, pulmonary abscess, bronchopneumonia or gangrene and these conditions may determine the character of the sputum.

Bronchopneumonia.—Bronchopneumonia may be due to a variety of organisms and occurs as a complication in many acute infectious diseases. It is a not infrequent complication of typhoid fever. It is very common in severe smallpox and is the rule in severe measles, diphtheria, whooping-cough and pulmonary influenza. It is the most common complication of typhus fever, in which disease it may result in gangrene, and is common in epidemic cerebrospinal meningitis. It is found in acute rheumatic fever, rarely in scarlet fever, and is the lesion in pneumonic plague. Under this title are included also hypostatic pneumonia and aspiration pneumonia. Children with bronchopneumonia often have no cough, more often raise no sputum; and this is true of some adults. The expectoration arises in both bronchi and alveoli and so is a mixture of rusty and mucopurulent sputum. Sometimes the transition from a bronchitis to a bronchopneumonia may be suspected from the changes in the sputum since it becomes less in amount, more viscid, more difficult to expel. It may be streaked with blood but it is practically never typically rusty.

In *Plague pneumonia* the consolidation is lobular and the sputum contains multitudes of Bacillus pestis. The expectoration may be scanty or abundant and when typical is liquid, not tenacious, frothy and bloody.

Influenza.—Pfeiffer described the characteristic sputum of influenza as greenish-yellow in color and expectorated in coin-shaped lumps. Other cases, he said expectorated a dark red, bloody sputum. For years the terms "influenza" and "grippe" have been interchangeably and carelessly used. According to some, grippe is the term which designates all epidemic colds with sudden onset and severe prostrating symptoms, whatever the bacteria involved, while "influenza" is the term reserved for any condition from which Bacillus influenzæ (see page 34) of Pfeiffer can be isolated. During the pandemic of 1918–20 the term "flu" gained considerable popularity and although at first an objectionable abbreviation yet may be the correct name of this epidemic since it has been widely used and of this epidemic only, while the use of the term influenza would imply that we consider this disease the same as the pandemic of 1889 and 1892 which probably is, but may not be, the case. This pandemic certainly was due to an, as yet, undiscovered organism.

At this point we would state the belief, which we accept, that the "flu" itself, a very contagious disease with leucopenia among its manifestations and due to an unknown invader, prepares the body for secondary infections, and perhaps even for a third or fourth "crop" of organisms, which are responsible for the marked and serious manifestations of the disease, and that it is this disease together with all its complications and sequelæ which has been called "influenza" or grippe.[58]

Belonging to the primary infection is a pharyngitis and bronchitis with a sputum devoid of characteristic features (except that when introduced into the throat of normal individuals does not communicate the disease) while the bronchopneumonia which often follows is in many cases (especially in the cyanotic cases with low diastatic blood-pressure) characterized by a very fluid sputum which consists largely of pure, bright red blood. In many other cases, however, the sputum of the pneumonia, usually lobular but sometimes lobar, is quite similar to other cases of these conditions.

These secondary invaders are, Bacillus influenzæ and the various streptococci, earlier the non-hemolytic and later the hemolytic varieties and the various pneumococci including Streptococcus mucosus *et al.* Bacillus influenzæ of Pfeiffer (1892) has attracted so much attention, for years considered the sole causative agent of influenza and now recognized as a very important as well as ubiquitous organism, that we describe it at length on page 34.

Whooping-cough.—During the catarrhal stage the cough is, as a rule, dry. A little later the sputum is that of bronchitis and presents no especial features. During the paroxysmal stage the sputum is expectorated in very small amounts each time and yet in the aggregate its amount is considerable. Bordet's bacillus, described on page 37, is now generally accepted as the cause of this disease.

Glanders of the Lung.—In case the glanders infection extends from the nose to the bronchi and there excites inflammation, a severe cough accompanied by a profuse purulent expectoration is the result.

Asthma.—In acute bronchial asthma the sputum may be quite characteristic. During the paroxysm itself there is often no sputum but expectoration begins while the paroxysm is subsiding, or, as the patient describes it, is "breaking," and brings much relief. But in more typical cases the patients expectorate during the paroxysm a tenacious, clear sputum containing thick, glairy, mucus balls, the so-called "perles of Laennec," which

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[53] See Lucke, Wight and Kline, Arch. of Int. Med., 1919, vol. xxiv, 154.

swim in a thin, clear, frothy mucus containing many coarsely granular leucocytes and many alveolar cells with myelin degeneration. In still other cases the sputum is less characteristic and consists of greenish-yellow tenacious mucus described by the patient as "rubber-like." It is common to find traces of blood in the sputum. These perles are pellets of a semi-transparent mucus, of a pearl-gray color like boiled tapioca, some of which contain mucous moulds of the smaller tubes and others Curschmann's spirals. The moulds are small cylindrical or sausage-shaped masses consisting of thick threads, or plugs, which may be from 1 to 1.5 cm. long. Some branch, some are narrow or straight, while others are spirally twisted. These last have the same significance as Curschmann's spirals. The amount of sputum at this stage may vary from a trace to 50 c.c. or even half a liter a day.

In 27% of the Johns Hopkins cases slight hemorrhages had occurred in at least 1 of the paroxysms. As the attack "breaks" the sputum becomes a clear viscid fluid, thinner, frothy, and more abundant, even 200 c.c. in 24 hours, and in it float mucopurulent masses, often spirals, and in 1 of our cases a true bronchial mucous cast about 1¾ inches long containing many coarsely granular cells.

During the next 2 or 3 days the character of the sputum changes much. It is often small in amount and mucopurulent, mixed with, however, some clear frothy fluid. As a rule, no Curschmann's spirals are then found, although in 1 case in which they had been abundant they were particularly beautiful. Fibrin casts of the bronchi sometimes accompany and may outnumber the spirals. Sometimes a branch of a cast ends as the central fiber of a typical spiral. With the spirals one also finds in the sputum many coarsely granular leucocytes, sometimes Mastzellen, often Charcot-Leyden crystals and calcium oxalate crystals.

The sputum usually ceases as soon as the attack is well over, but it may continue, even 100 c.c. per day, or recur at intervals separated by sputum-free periods.

Curschmann's Spirals.—These beautiful structures (see page 7) probably appear at some time or other in the sputum of every case of true bronchial asthma, but certainly not with every paroxysm. We have in mind a man whose sputum furnished the students of several years ago with an abundance of beautiful spirals, but during the past 15 years although admitted to the ward 14 times during acute attacks of asthma, only once was a spiral found. While these spirals may appear at any part of the paroxysm they are most numerous just at the end, in the clear mucous sputum, and disappear after the sputum becomes mucopurulent.

Charcot-Leyden Crystals.—Charcot-Leyden crystals (see page 18), found wherever eosinophile cells are numerous, are present in abundance in the sputum during attacks of asthma. They may occur in groups visible even to the naked eye as specks of a greenish-yellow color, which color they may give to the spirals. Their size varies so much that their presence can be excluded only after a search with the oil immersion lens. Their size and number in the sputum increase as the attack of asthma continues, but they often can be found only after the sputum has stood in a thermostat. These are much more constant in the sputum of asthma than the spirals and do not necessarily indicate the presence of an eosinophilia of the blood.

Alveolar epithelial cells laden with golden-yellow pigment, similar to the Hertzfehlerzellen of chronic passive pulmonary congestion, may appear in large numbers in definite masses in the sputum in asthma. They may fill a considerable part of the mantle of a spiral.

Certain cases would seem to represent a transitional stage between asthma and fibrinous bronchitis, since their sputum contains spirals, Charcot-Leyden crystals and eosinophile cells, but also casts of the smaller bronchi, the tips of whose branches may be directly continuous with the central fiber of a true spiral. In one case of Dr. Osler's [59] already mentioned, these casts were 1 to 3 cm. long.

Acute Bronchitis.—Acute bronchitis is a diagnosis frequently and yet often erroneously made. It should be used of an acute infection of the mucosa of the bronchial trees and yet in most of the cases thus named the inflammation seldom extends far down the trachea while in those with a definite acute bronchitis this often is overlooked since it is a minor feature of a general systemic infection. Another reason for confusion is that with an extensive acute bronchitis there probably always is some definite bronchopneumonia. Acute bronchitis is common as a manifestation of the infection of influenza, measles, typhoid fever, meningitis, whooping-cough, syphilis, typhus fever, smallpox, etc., and in these conditions a bronchopneumonia also is sometimes present. In these cases the bronchitis may be due to the organism of the general infection but more often it is a complication due to one of the streptococcus or pneumococcus groups whose presence is considered an illustration of symbiosis. Among the organisms found in the bronchial secretion are: Bacillus influenzæ, Micrococcus pneumoniæ, members of the staphylococcus group and streptococcus groups; Micrococcus catarrhalis, as well as the organisms of the specific infections mentioned above, Bacillus typhosus, Diplococcus meningitidis, etc.

In simple acute bronchitis there is often no sputum at all, but if present that at the onset is very scanty, frothy, transparent, tenacious, very hard to expectorate, the "sputum crudem," of older writers. It consists of almost pure mucin, enclosing a few leucocytes, red blood-cells and a few bronchial epithelial cells, some ciliated, some even with the cilia in motion. The few mononuclear leucocytes present, the so-called "mucous corpuscles," are perhaps derived from the lymphatic masses along the respiratory tract. In some cases the sputum contains so many alveolar epithelial cells that the condition has been named "desquamatory bronchial catarrh." Myelin drops are found, but only the simpler forms and they are not very numerous. Such sputum is due to an hypersecretion of the mucous glands, together with the desquamation of a few epithelial cells. While the sputum may continue of this character during the whole course of the acute bronchitis, yet as a rule it later becomes mucopurulent. In some of our cases it was 2 weeks before very much pus appeared. In others it is at the onset more watery and so is termed "seromucous" sputum (Biermer). In some cases the sputum is quite bloody at the onset of the attack. This was true in 33% of our cases.

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[59] See Bettmann, Amer. Jour. Med. Sci., February, 1902.

After the first 2 days or more the cough usually "loosens," the sputum increases in amount, becomes less viscid, less tenacious, and may resemble the white of an egg, since it is frothy and shows whitish streaks. Sometimes it is blood-streaked.

The sputum later becomes mucopurulent. It contains all of the elements mentioned above, but with the pus-cells very much increased. These may be uniformly distributed and give the sputum a uniform yellow color, or they may be present in islands. There are still many epithelial cells present but these have lost their shape and their cilia, are now round and often fatty. Such sputum was formerly called "sputum coctum."

In a typical case the sputum next becomes almost pure pus which pours from the inflamed and partially denuded mucosa. It is opaque yellow or a yellowish-green and is often expectorated in masses. The amount as a rule varies from 100 to 200 c.c. in 24 hours, and most in the morning. Microscopically the leucocytes are nearly all polymorphonuclears although a certain number are mononuclears. No cylindrical cells can now be found. Alveolar epithelial cells, some containing pigment and some fat granules, may be found if searched for. It contains also much mucus, much myelin and fat globules often in masses which in shape suggest cells. In certain cases the sputum contains a surprising amount of fat, some in cells, some in free droplets but more in the above-mentioned masses of droplets. In other cases very little fat is found. The reason for this difference is not known (Hoffmann).

As the case improves the sputum becomes more abundant, more purulent and less tenacious. It then, as improvement continues, diminishes progressively in amount and finally ceases.

The above is the sequence in a quite typical case. The following variations however are met with. In 13% of our cases, the diagnosis of acute bronchitis was made because of the physical signs and no sputum was at any time obtained. In other cases it was so tenacious that it could scarcely be expectorated, the patient often vomiting in the attempt. In some cases it was mucopurulent and fairly abundant from the onset but these cases probably were acute exacerbations of a slight chronic bronchitis, since over 50% of them stated they had been subject to coughs and colds, while that history was obtained from fewer than 20% of those whose illness began with scanty expectoration.

In about 35% of our cases whatever sputum there was was viscid, very tenacious and scanty and was followed by a period with a dry cough. In about 10% the sputum at the end of the attack consisted of a watery serum in which floated islands of mucus and pus about 1 cm. in diameter, which settled to the bottom of the cup. Other cases were interesting in that the sputum at the end of the attack was similar to that of the beginning, *i.e.*, was a pure mucus.

In the so-called *capillary bronchitis*, *i.e.*, an acute bronchitis of the smaller bronchi, the cough is frequent, often paroxysmal and at first dry. There may be no sputum throughout the entire course of the disease or it may be scanty and expectorated with great difficulty. In these cases a diminution in its viscosity is a sign of improvement.

The acute bronchitis of early typhoid fever deserves mention since it is so constant as to give rise to the adage, "If no bronchitis, it is not typhoid fever." The sputum usually is mucopurulent and contains Bacillus typhosus in pure culture or with Micrococcus pneumoniæ, one of the streptococci, or Bacillus influenzæ.

In pulmonary anthrax, or wool sorters' disease, the condition is usually one of bronchitis although pneumonia may develop.

Chemical Analysis.—The chemistry of the sputum in acute bronchitis is of very slight interest. Of the cases which have been reported by Bamberger, Biermer, and Renk, the water content has varied from 95.62 to 98.3%; the organic substances from 1.17 to 3.7% and the inorganic salts from 0.457 to 0.76%.

Chronic Bronchitis.—Under the heading chronic bronchitis may be included all cases of bronchitis from the simple subacute type of a cough which has merely "held on" to those cases which give a history of cough with expectoration extending over 25 or more years. These long standing cases are usually kept alive by focal infections in the nose, tonsils or mouth. The subacute cases expectorate for weeks or months a sputum which is scanty, tenacious and viscid. Some patients describe their sputum as consisting of thick leathery lumps; others, as a white, sticky mucus. Later on it usually becomes more abundant and mucopurulent and hence yellower. Some patients for weeks expectorate an abundant sputum with a dark greenish color and a foul odor, which tends to separate into 3 layers: a mucous layer, a brownish-gray serum and a mucopurulent sediment. The sputum in these cases gradually diminishes in amount until the patient is apparently well, but he certainly is susceptible to other and similar attacks.

The bacteriological examination of the sputum of these cases usually shows a mixed infection with 2 or more organisms, among them Bacillus influenzæ, Micrococcus catarrhalis, the pyogenic cocci, pneumococci, Bacillus mucosus capsulatus, and Streptococcus capsulatus. Of the comparatively pure infections that of Bacillus influenzæ is apparently the most common, but repeated examinations over long periods usually show that one group of organisms does not long remain unmixed in the sputum (Lord).

Patients admitted to our hospitals for acute bronchitis usually have acute exacerbations of a chronic bronchitis. That is, they have had for a long time a dry cough which now has given place to one with sputum, or they have had a chronic cough with scanty sputum and now expectorate an abundant mucopurulent, often blood-streaked sputum.

During an acute exacerbation of a chronic bronchitis the sputum is sometimes scanty, very tenacious and purulent; sometimes abundant, mucopurulent and but slightly tenacious; while in other cases, and these are perhaps the most common, is abundant, white, frothy, seromucous and contains very little pus. In still other cases the sputum will be very large in amount but homogeneous and extremely viscid, a single, purulent, glutinous jelly-like mass filling the cup. Its odor is sometimes foul, in one of our cases almost putrid. The amount may vary from 100 to 200 c.c. in 24 hours. Later the sputum increases in amount and contains small mucopurulent flakes. Such sputum separates into 2 layers, a serum above and a layer of the solid flakes below. In other cases there is a tenacious green mucous layer above and a fluid layer below.

The most common type of chronic bronchitis is the so-called "winter cough" of patients who during the summer are apparently well. This bronchitis may recur for 20 winters or more and yet as a rule the cough soon becomes continuous throughout the year. Such cases expectorate chiefly in the morning, and describe themselves as then "clear" for the day. Some of these patients expectorate each morning about an ounce of mucus, while others raise thick yellowish masses of sputum. In severe cases the cough is paroxysmal and the sputum sticky, frothy, sometimes blood-streaked and very hard to raise. In some cases of chronic bronchitis the sputum during an acute exacerbation becomes more scanty and more tenacious than before rather than more abundant. These patients feel better when their cough loosens.

Dry Catarrh.—The "*catarrhe sec*" of Laennec is a symptom-complex much in dispute, but cases of chronic bronchitis with little or no sputum are not rare. According to English authors, this occurs particularly in "gouty" patients. We associate it, however, more with emphysema and myocarditis. Many of these do raise a little which is glutinous and pearly.

The chronic bronchitis which accompanies emphysema of the lungs is an especially common form. For instance, of 100 cases of long-standing bronchitis, in 43 pulmonary emphysema was a marked clinical feature. Of 100 cases of pulmonary emphysema 58 suffered also from bronchitis, and 47 of these from long-standing bronchitis. This cough at first recurs each winter, the patient is comparatively free in summer although later it is apt to become continuous. Of these patients with chronic bronchitis 11% claimed to expectorate no sputum whatever at any time. In most of the cases with scanty sputum the expectoration for years occurred only in the morning, and consisted for the most part of a slight amount of bluish-white tough mucus. It may, however, be large in amount. One patient, for instance, for years awakened at 5 o'clock each morning with a severe paroxysm of coughing and expectorated a large and almost solid mass of thick mucus. In other cases the sputum is abundant, even 1 pint a day, frothy and whitish in color. In our cases of emphysema with chronic bronchitis and admitted during an acute exacerbation of the bronchitis the changes in the sputum due to the acute exacerbation varied much. As a rule its amount increased very materially. In one case it became putrid. In one-fifth of the cases it was blood-streaked. As these cases improved the sputum first became still more abundant, white and frothy, and then gradually diminished to the previous condition.

In the sputum of 2 cases there were a great many eosinophile cells. Some sputa contained large amounts of myelin and others large masses of fat globules.

One patient with chronic bronchitis had had for 10 or 12 years a slight expectoration, but on admission his sputum was abundant, thin, cloudy, and contained moulds of the bronchi, the stem of some 0.5 mm. in diameter, which consisted of mucus, pus and alveolar epithelial cells. This sputum also contained much pigmented alveolar epithelium, pus-cells and red blood-cells.

One emphysematous patient with long-standing bronchitis, admitted during a rather acute attack with continuous fever, expectorated sputum which was abundant, seromucoid, never bloody and which contained during repeated examinations large numbers of sarcinæ.

In 1 case of chronic bronchitis with emphysema and "hay fever" the sputum was yellowish-green, mucopurulent, slightly blood-tinged and contained branched bronchial plugs which consisted of mucus, pus-cells, many eosinophile cells and masses of the mycelial threads of some mould.

In the *chronic bronchitis of mitral cardiac disease* the characteristic sputum contains a large amount of blood, which gives a bright-red or prune-juice color. In other cases, particularly of mitral stenosis, it is often stained by masses of Hertzfehlerzellen. Other cases, however, expectorate merely large amounts of frothy, seromucous pus.

BRONCHORRHEA.—If by bronchorrhea one means, with Laennec, a chronic idiopathic disease characterized by the expectoration of large amounts of watery sputum we may doubt the existence of such a disease. But if we mean merely chronic bronchitis with an abundant watery sputum the condition is by no means rare. Some have described a "bronchorrhea serosa," or "asthma humidum," with abundant, very watery, colorless, foamy sputum. Some of these cases are said to have a neurotic basis. In the bronchorrhea of chronic bronchitis the patient may expectorate about 500 c.c. a day which is as a rule purulent, watery and of a green or a yellowish-green color. In cases of "bronchoblennorrhea," with bronchi denuded of mucosa and lined by a pyogenic membrane, the sputum contains very little mucus, is a profuse watery pus which separates easily into 3 layers and which may have a very bad, although not a distinctly fetid, odor. Sputum somewhat similar is seen also in bronchiectasis and perhaps also in cases of putrid bronchitis and lung gangrene.

PUTRID BRONCHITIS.—In many cases of chronic bronchitis the sputum has a disagreeable, almost fetid, odor; but in putrid bronchitis it is truly fetid. Fetid sputum occurs also in many cases of bronchiectasis, in gangrene of the lung, in abscess, in tuberculosis with large cavities and in empyema perforating through the lung. Cases of fetid bronchitis with the bronchi not dilated certainly are very rare (Fowler and Godley), some even deny that they exist (Hoffmann) and claim that these were cases of bronchiectasis, which in time any case of putrid bronchitis would soon become. A very few genuine cases of putrid bronchitis have, however, come to autopsy (Osler).

The sputum in putrid bronchitis is profuse, watery, of a dirty ash-gray or a brownish color, and with a horrible odor which will fill the whole house. Allowed to stand, it separates into an upper layer of frothy air-containing mucus, usually small in amount since the mucous membrane is for the most part destroyed and from which layer stream downward brownish strands; a middle layer of serum, and the lowest a thick sediment of epithelial cells, fatty cells, free fat, almost pure pus, all kinds of bacteria and sometimes Dittrich's plugs. No elastic tissue fragments of lung are to be found.

Gangrene may follow in such cases.

Chemically one finds in the sputum of these cases many of the products of the decomposition of proteids; volatile acids, among them butyric and valeric; NH_3, H_2S, leucin, tyrosin, etc.

Fibrinous, Croupous, or Plastic Bronchitis.—The acute form of fibrinous bronchitis which accompanies certain infectious fevers is mentioned on page 9. Chronic idiopathic fibrinous bronchitis is so rare a disease that Bettmann could find but 27 cases in the literature of 35 years. During an attack of fibrinous bronchitis the sputum, for 5 or more days, consists merely of abundant mucus and then suddenly there develops a severe coughing spell and the patient expectorates a bronchial cast. Blood usually accompanies the cast but may precede or follow it. Profuse hemorrhages are rare. The frequency with which casts are expectorated varies much. Usually but one is expectorated during several months but some patients expectorate 1 every 2 or 3 days while 1 patient expectorated 3 during the same day. The casts appear in the sputum as formless masses, but if shaken out in water are found to be moulds of a bronchial tree. All from the same patient are apt to be exactly alike as though all came from the same bronchus. Some would seem to represent the bronchial tree of an entire lobe. The largest casts are about 10 cm. long, are grayish-white in color, contain a great many air bubbles and in about one-third of the cases are blood-streaked or contain a blood-clot in their lumen. They consist of a fibrin-like material in layers arranged concentrically, the innermost of which presents many whorls, since this was the first formed and this is much compressed by the layers peripheral to it and formed later. The casts are loose in structure, are usually hollow, although some are solid; of others the larger branches are hollow and the smaller solid, while in others the reverse is true. In the central layer, the oldest, are seen the remains of cells, alveolar and bronchial epithelium, leucocytes, red blood-cells and bacteria, but in general these casts contain much air and few cells. Sometimes the casts contain much fat as does also the sputum.

Casts are not always products of the epithelial cells of the mucosa. There was, for example, no epithelium in that part of the bronchial tree from whence the casts came in the case of the above-mentioned patient who expectorated 3 in 1 day. Those casts must therefore have been the result of direct exudation.

Bronchial casts were formerly supposed to consist of fibrin, since their physical appearance suggests this. Others claim it is mucus, others say syntonin or coagulated albumin. This material sometimes takes Weigert's fibrin stain, but usually it does not. Liebermeister [60] reviewed this question at length as the result of the study of 1 fresh case and of 12 museum specimens. He demonstrated fibrin and mucin in 7 of these 13 cases. To demonstrate fibrin he used Kockel's method, and thionin for the mucin.

Charcot-Leyden crystals are commonly present in these casts. In the same sputum spirals also sometimes are found. In Vierordt's [61] case there were many such casts, and on one occasion a typical Curschmann spiral. In Dr. Osler's case, mentioned by Bettmann, the ends of some branches of the casts were directly continuous with the central threads of true spirals. Many eosinophile cells also are present, also red blood-cells, hematoidin crystals, and lecithin granules.

Casts similar in appearance appear in the sputum of other conditions: in diphtheria, which casts are firm hollow tubes of dense fibrin enclosing countless cells; those in pneumonia are mentioned on page 63; and the cast from 1 case of heart disease was similar to those of fibrinous bronchitis.

Syphilis of the Trachea and Bronchi.—In syphilis of the trachea and bronchi the cough is at first dry but later is accompanied by mucoid, mucopurulent, or purulent sputum which often is blood-stained and which sometimes contains also elastic tissue. Hemoptysis has been a prominent feature in about one-half of the cases, while about half of the fatal cases have died from hemorrhage from ulceration of the luetic lesion of the trachea or larger bronchi through into the pulmonary artery, aorta, bronchial artery or superior vena cava.

Bronchiectasis.—The sputum in the saccular form of bronchiectasis may be quite characteristic; in the diffuse form it is practically never so. The former is marked by 2 features—its large amount and its periodicity. That is, the patient occasionally, and usually following some definite change of posture, will have a paroxysm of coughing, often severe, and expectorate a large amount of sputum. Having emptied the sac of the dilated bronchus he may for hours be free from cough and sputum. Since this follows a marked change in the position of the body, it most often occurs on rising in the morning, but the posture which will liberate the coughing reflex will depend in a large measure on the position of the sac. This periodic feature was marked in but 10 of our 24 cases. The amount of sputum these patients have is considerable, as a rule from 750 to 900 c.c. in 24 hours, but in 1 of our cases it frequently exceeded 1 liter. Such profuse expectoration may extend over a considerable period of time.

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[60] Deutsch, Arch. f. klin. Med., 1904, Bd. 80, 5, and 6.
[61] Berl. klin. Wchenschr., July 16, 1883.

Of 23 cases, in 2 the sputum for 24 hours was under 100 c.c.; in 11, from 1 to 300; in 2, about 500; while in 7, it exceeded 600 c.c. In general the amount of sputum bears but little relation to the duration of the disease. One of our cases, of 26 years' standing, expectorated only from 15 to 30 c.c. a day. It bears little relation to the size of the cavity, as was shown by 1 of our patients who expectorated more than 1 liter of sputum each day and yet at autopsy a few, surprisingly small, cavities were found. It is stated that there is a remarkable diminution in amount of sputum as the patient grows weak before death.

The most characteristic sputum in bronchiectasis is grayish or grayish-brown in color, fluid, purulent, of a disagreeable odor and separates on standing into 3 layers. But this is not the only form. Early, while the bronchiectatic cavity is lined by mucous membrane, the sputum is a pure, clear mucus, but soon the cavity becomes infected and then the mucosa becomes a pyogenic membrane which produces a yellow, purulent fluid with a sweetish odor. Sooner or later putrefactive organisms invade the cavity and then the sputum becomes of any shade of gray or green, mucopurulent, and has a horrible fetid odor. Those of our cases with the worst odor were dirty gray in color. There is usually bleeding into the cavity and then the color of the sputum may have any shade of red or brown according to the chemical changes which the hemoglobin undergoes. While, as a rule, the sputum is very fluid and watery, in some cases it is thick and viscid, while in other cases it is mucopurulent and contains masses suggesting nummuli. In certain cases well illustrated in our series, particularly those which improved under treatment, the sputum, which was at first profuse and watery, later diminished in amount, became mucopurulent and of a less offensive odor. The tendency to form 3 layers on standing in a tall glass vessel was marked in 14 cases. These layers are: an upper frothy mucous layer, a middle serous, and a lower, always thick, granular layer. From the upper layer often hang down through the fluid "streamers" of mucus laden with pus. This is often spoken of as the second layer. Hoffmann and others mention but 2 layers, omitting the upper which was absent in 3 of our cases.

In 4 of our cases there were 4 well-marked layers; the lowest, abundant, of greenish-red purulent material; the one above, containing a good deal of blood and hence red or brown; over this a serous layer and on top a frothy mucous layer. In other cases below the top layer was a mucopurulent layer of streamers hanging down through the fluid, which with a little encouragement would probably all have sunk to the bottom. The odor is, in general, bad, but in 2 of our cases it was not at all offensive. In some cases it had at first no odor, then became slightly offensive, while later, after the putrefactive changes had set in, it was fetid. These changes are due to secondary infections of the cavity. In 10 of our cases the odor was heavy and sweet, while in 10 others it was at some time very fetid. This fetid odor is not exactly the same as that present in gangrene and has been described as "pseudogangrenous" in character, the odor of rotten cabbage, or garlic. This odor will often diminish after long-continued treatment with creosote inhalations or intratracheal injections. Indeed a patient admitted with extremely fetid sputum may leave the hospital with expectoration much reduced in amount and not at all offensive in odor. The breath of the patients is sometimes worse than their sputum. The disagreeable odor is largely due to H_2S, NH_3, and various volatile acids, among which are acetic, butyric, and formic acids.

Hemorrhages into the bronchiectatic cavities are common, occurring in even 50% of all cases (in 17 of our 24 cases). The hemorrhage is slight as a rule (8 of our cases) but sometimes (6 cases) is considerable in amount, while in 3 it was extreme. In some cases it has been fatal.

One of our cases was admitted to the hospital 14 times, 5 because of extreme hemorrhages which threatened his life. During one of these admissions he had, in a very few days, 6 large and several smaller hemorrhages, which reduced his blood count rapidly from about normal to 1,090,000 red blood-cells and the hemoglobin to 20%. Another case in 1 day and in about 10 minutes lost 1700 c.c. of blood and on the following day died from a hemorrhage.

Microscopical Constituents.—As long as a bronchiectatic cavity is not infected its contents will consist of mucous and desquamated epithelium cells. After infection its wall becomes a pyogenic membrane and its contents those of an abscess. Later, infection with the organisms of putrefaction is the rule. The pus-cells, enormous in numbers, are well preserved, fatty, or vaculated. The red blood-cells are unchanged or very much altered. Elastic tissue theoretically should not be found and yet it was present in 2 of our cases, indicating ulceration of the bronchial walls. Fatty acid crystals are numerous especially when the outlet of the cavity is small, thus allowing considerable stagnation of its contents. These crystals occur in large masses, are very large in size and present a beautiful picture. They were abundant in 4 of our cases (see Fig. 8). Cholesterol is often present; hematoiden crystals frequently; leucin and tyrosin sometimes; and Dittrich's plugs, usually. Alveolar epithelial cells are often present, some containing pigment and others much myelin and fat. No tubercle bacilli are found, but other bacteria are, in great numbers and form large zoöglea. Yeasts are found, and, in one of our cases, an aspergillus mould. Calcium salts are sometimes deposited in the contents of these cavities, giving rise either to a clay-like mass or as in 2 of our cases to a bronchiolith. In 1 of these cases, that of a man who during life has expectorated several concretions, the pathologist found at autopsy considerable calcareous matter embedded in the walls of a cavity. The bronchioliths in his sputum were about the size of split peas.

In other cases, as in 13 of our series, the sputum is by no means so characteristic and resembles that of a chronic or of a fetid bronchitis

Children with bronchiectasis are apt to swallow and then to vomit their sputum.

Gangrene of the Lung.—The most characteristic sputum of patients with pulmonary gangrene is profuse in amount, watery, greenish-brown or ashy-gray in color, has an extremely fetid odor and separates easily into layers. But more commonly it contains blood and so has a color which may vary from reddish-brown to brownish-red or even chocolate. In other cases it has a uniform dirty-brown color due to masses of hematoidin crystals. Its odor usually is the worst of all the sputa and yet in some cases of true gangrene of the lung the sputum and the breath have no odor at all. In 5 of 12 cases of the Johns Hopkins Hospital the presence of the gangrene was not even suspected during life. One case had expectorated merely "phlegm." These patients are usually diabetics or insane. In cases of pulmonary embolism the infarcted area may become gangrenous and later be evacuated through a bronchus.

It is often difficult to differentiate between gangrene and abscess of the lung, since whichever is primary the other is quite sure later to develop. The presence of tissue fragments will aid in the differential diagnosis between gangrene and long-standing bronchiectasis. Putrid bronchitis differs from gangrene only in the absence in the sputum of fragments of lung tissue. This sputum separates easily into 3 layers—the upper of frothy mucus, the middle of serum and the lowest, always a thick one, of pus, tissue detritus, Dittrich's plugs and tissue fragments. From the top layer streamers often extend down through the fluid. In other cases, the sputum is mucopurulent in nature, while in others it is viscid, lumpy, mixed with blood and also very fetid.

Of the macroscopic constituents of the sputum of cases of pulmonary gangrene the fragments of necrotic tissue are the most important and must be demonstrated before a positive diagnosis is possible. It is in this disease that the largest fragments of lung are expectorated. Some are very minute but others are even several centimeters in length. Some are firm in texture, sooty in appearance, have ragged outline, while others consist of a colorless ground substance full of granular detritus, fat droplets, clumps of coal, large fat needles, bacteria and elastic tissue. The other constituents of the sputum are those of fetid bronchitis.

It is said that elastic tissue is rarely if ever found in the sputum of this disease and that its absence is due to some ferment which digests it in the tissue masses. The chances are, however, that it can always be found if searched for. To be of aid in diagnosis the elastic tissue must show by its arrangement its origin in the alveolar walls. Alveolar epithelium, often pigmented, is easily found; fatty acid crystals and fat droplets are abundant; cholestrol, leucin, and tyrosin may be demonstrated; masses of bacteria and often of leptothrix are conspicuous, while flagellata have been described. In a case mentioned by Sahli, in which the infected area was non-odorous, large numbers of sarcinæ were found. Blood is frequently present and in large amounts, the origin of which is the rupture of small vessels, not diapedesis. Fresh blood was present in 5 of our cases, but as a rule the hemoglobin is present as methemoglobin and hematin.

Many observers (*e.g.*, Mayer) in 10 of 58 cases have found acid-resisting but not alcohol-fast organisms in the sputum of these cases (see page 30).

Aspirated Foreign Bodies.—The aspiration of a foreign body immediately gives rise to a paroxysm of cough, suffocation, cyanosis and intense dyspnea. If the foreign body remains impacted this soon subsides and a period of comparative relief may follow which often leads to a false sense of security marked only by occasional slight cough and dyspnea on exertion. In a few days, however, the manifestation of secondary bronchial infection appears, *i.e.*, cough, with more or less abundant sputum which is purulent and soon foul-smelling, then the clinical features of pulmonary abscess develop which may last for weeks, months, or even years.

Abscess of the Lungs.—Abscesses of the lung are evidently much more common than was believed. Indeed they may follow any operation on an infected tissue, even simple tonsillectomy, and may be a sequela of any bronchopneumonia due to a pyogenic organism, as the so-called ether pneumonia, metastatic pneumonia, etc., as well as a common sequela of influenza. They may rupture into a bronchus causing no marked symptoms if small, or into the pleural cavity causing empyema. An abscess of the lung may be suspected if the patient suddenly expectorates a large amount, even several hundred cubic centimeters, of quite pure pus in which are fragments of lung tissue. If allowed to stand this sputum will separate into several indefinite layers, but layer formation is not definitive since a slight shaking will restore the sputum to its previous homogeneity. The odor of this sputum is at first faintly sweet like all pus, but if lung gangrene develops as it often does, it becomes foul. The lung-tissue fragments are very important in diagnosis. In size they vary from about that of a millet seed to fragments even 2 inches long. In the larger fragments of lung tissue may be demonstrated a framework of elastic tissue, the remains of blood-vessels, masses of coal dust, fat crystals, free fat, detritus, hematoidin crystals, amorphous clumps of pigment, and zoöglea of cocci. In other cases one finds no large fragments. In these the lungs undergo the so-called "insensible disintegration" (Leyden). In this sputum one finds separate elastic fibers and free cholesterol, fatty acid crystals, free fat, lung pigment, detritus, bacteria and hematoidin crystals which may be present in large numbers and give to the whole mass of sputum a brown color. Older writers have described a sputum the gross appearance of which was quite characteristic of lung abscess. If one shakes it out in water it would appear like a skein or thread of pus. The explanation of this would seem to be that the pus escaped in a thin thread from a large cavity slowly through a small opening and later received in the bronchi a mucous coating which prevents its coalescence. We have seen no such case.

The sputum of a *liver abscess perforating through the lung* is often characteristic because of the bitter taste of the bile acids, its so-called "anchovy-sauce" appearance, or its ocher-yellow color from bile pigment. In some of these cases a lung abscess also develops, in other cases only a simple hepaticobronchial fistula. Microscopically bilirubin crystals and much elastic tissue will be found and sometimes the amebæ themselves.

In the records of the Johns Hopkins Hospital there were 7 such cases. In 3 the sputum was abundant, even over a liter in 24 hours. In some the expectoration was paroxysmal, even a quart at a time. The odor was mildly offensive in 2 and markedly so in 2 others. In six of these cases the sputum presented the typical "anchovy-sauce" appearance; that is, it was of a rusty brownish-red color and frothy. In 4 cases it was blood-streaked and in 2 purulent. Microscopically were found the ordinary elements of sputum: pus, red blood-cells and alveolar epithelial cells, and, in addition, fat crystals and the crystals or needles of hematoidin (bilirubin) which were a marked feature in 2 cases. Elastic tissue was found in considerable amounts in 5 cases. In 2 cases the liver cells, it was thought, could be recognized. The living active amebæ were found in the sputum of 5 cases, on 1 even before they were found in the stools. The sputum which contained the ameba also contained much elastic tissue. If the sputum be preserved in the thermostat the ameba will remain alive and motile for even a whole day.

The influenza epidemics were followed by many cases of lung abscess, the logical results of the areas of interstitial pneumonia. We believed that many of the cases of empyema were due to the rupture of 1 of these abscesses into the pleural cavity.

E. S., No. 7554, a boy aged 16, was admitted Dec. 15, 1918, following an attack of influenza which began Nov. 21, 1918. On December 16 he suddenly coughed up 300 c.c. of thin, purulent sputum with a sweetish yet fetid odor. Smaller amounts followed. On standing this sputum separated into 3 layers, the upper of mucus, the middle a thin turbid grayish liquid and the lower which was thick, ropy and salmon colored. Four days later 1600 c.c. of foul smelling thick pus were aspirated from the right chest. This did not separate into layers on standing and contained many streptococci. The left lung and pleural cavity were quite normal throughout. He was discharged well March 28, 1919.

ABSCESS OF THE LUNG FOLLOWING ACUTE LOBAR PNEUMONIA.—In 3 of 6 cases of fatal pneumonia with pulmonary abscess (found at autopsy) there had been no clinical features which suggested this complication. In these cases the abscesses were small and multiple and there had been little or no sputum. In 1 of these cases the only change noted in the sputum was that the viscid, tenacious, blood-streaked expectoration became less tenacious. In 1 case the sputum, which had been small in amount, very tenacious and blood-tinged, suddenly became very dark brownish-black in color and mucopurulent. It then became greenish in color and small in amount. Then all expectoration stopped, soon to begin again as a mucopurulent sputum. It then became very green and scanty and later increased much in amount, was very thick, very purulent and of a sour odor. It then became thinner, more watery, blood-stained and contained elastic tissue. Then it decreased in amount, became mucopurulent, and finally, with the recovery of the case, ceased. In 1 case large numbers of trichomonads were found in the sputum.

Three cases of *post-operative pulmonary abscess* were followed clinically. One patient who was admitted with a paroxysmal cough suddenly expectorated a large amount of foul-smelling sputum. The odor later became less offensive. It contained pus and fatty acid crystals. This sputum contained large fragments, even 5 by 3 cm. in size, evidently of tissue but so decomposed that their structure could not be determined. The sputum then became less profuse, then mucopurulent and the patient recovered. In another case the sputum was very foul-smelling and contained much fat, while in another case it was large in amount, purulent, foul-smelling and blood-streaked. Of 2 other cases not recognized during life 1 had showed a sudden increase in the amount of sputum, while in the other an abundant, blood-streaked, brownish sputum of no especial odor suddenly increased in amount, became dirty, frothy, foul-smelling, slightly blood-streaked and separated easily into 3 layers. At autopsy a large abscess cavity was found.

Perforating Empyema.—The sputum of cases of pleural empyema which perforates through the lung resembles that of abscess of the lung, with the exception, however, that that of the former contains less elastic tissue and practically no tissue fragments. It contains many hematoidin, as well as other, crystals. Its odor is at first that of pus, in some cases described as resembling old cheese, but later it becomes vile because of the secondary infections which commonly follow. In case the pleural fluid escapes slowly through a small pleurobronchial fistula, it is said that a skein of a thread of pus may form similar to that described on page 78. When the opening is large the pus will escape rapidly, yet without causing pneumothorax. Allowed to stand, this sputum separates into 3 layers, the upper of mucus, the middle of the pus-serum and the lowest of pus-cells.

Perforating Serous Pleurisy.—It is exceedingly rare for a serous pleural effusion to rupture through the lung. The sputum in such a case is like that of edema of the lungs, but contains more albumin and so becomes quite solid on boiling.

The Serous Sputum of Edema of the Lungs.—Patients with edema of the lungs expectorate large amounts of a frothy, cloudy, colorless, or a slightly bloody sputum which on standing separates into 3 layers: an upper abundant frothy layer, a middle foamy fluid, and a thin lower layer of pus together with the elements of the pre-existing sputum. Excepting in cases of pneumonia, etc., the sputum is quite pure blood-serum. It is frothy since it is so rich in albumin and watery since it contains only a trace of mucin. Patients during the last hours of life with this sputum flowing in continuous streams from their nostrils and mouth present one of the most gruesome sights of the wards.

The Albuminous Expectoration of Thoracentesis.—Among the recent important articles on albuminous expectoration are those of Riesman[62] and of Allen.[63] Terrillon has grouped cases of this condition into 3 classes: The first of mild cases with sputum varying in amount from little to 800 c.c.; the condition of these patients is always good. The severe cases have dyspnea and collapse, and expectorate from 1200 to 1500 c.c. In the grave cases the fluid may suddenly gush from the mouth. Some patients are drowned in the fluid which they cannot expectorate rapidly enough, while others have died before they could expectorate any.

The expectoration of this albuminous sputum may begin with a paroxysm of coughing during aspiration, or in less than 1 hour after this is finished, while in the latest cases it begins 18 hours after the tapping and continues for 24 hours. As a rule it lasts from 1 to 2 hours. This sputum is rich in albumin, frothy and neutral or faintly alkaline in reaction. To test it for albumin the sputum should be diluted and filtered and the filtrate tested by heat and nitric acid, by nitric acid alone, or by potassium ferrocyanide. Acetic acid without heat gives a precipitate of mucin. This sputum also contains urea, hemaglobin, urobilin and the various salts of blood-serum. The amount expectorated averages from 200 to 900 c.c. while even 2 liters have been expectorated. On standing 3 layers separate—the upper, whitish and frothy, the middle, opalescent and yellowish or greenish and the lower, more viscid, contains a few whitish flocculi and sometimes slight traces of blood, but rarely much. In Riesman's case there was no lower layer, the specific gravity was 1.018, the fluid became solid on heating. The total solids were 5.84%. In Allen's case the expectoration began in half an hour after 3100 c.c. of pleural fluid had been removed and lasted 4 hours. It measured about 1 liter in amount, was frothy, pale green in color, with a muddy sediment. Microscopically were found flat epithelial cells, a few leucocytes, red-blood cells and many bacteria. Other such sputa have had quite different composition. Some resembled pleural exudates.

The cause of albuminous expectoration has been in much dispute. The majority of writers think that it is due to an acute edema of the lungs and is the result of too rapid or too complete thoracentesis. We would call attention, however, to certain cases which occurred during thoracentesis and which were followed by pneumothorax. In these cases the pleural exudate itself may have been evacuated through the mouth.

Hemoptysis.—For the causes of pulmonary hemorrhage we quote both Osler and Lord.

Hemoptysis may occur: (1) In young healthy persons, without known cause and without subsequent symptoms. (2) As the first symptoms of pulmonary tuberculosis. This explains 77.6% of the cases of sudden hemorrhage in healthy young men and is due to mucous erosions and to the diapedesis of red cells through the congested mucosa. Sudden hemorrhage was noted in 900, or 0.045%, of the soldiers of the Prussian army studied with this point in view, all apparently healthy young men. In 480, or 54%, there was at the time no apparent cause. Of these 480, 417, or 86%, were probably tuberculous and 221, or 46%, quite certainly so. (3) In an advanced case of pulmonary tuberculosis or in a cured case with cavity formation, due to the rupture of a miliary aneurism on a branch of the pulmonary artery exposed by cavity formation. (4) In other diseases of the lungs, and this list includes practically all pulmonary diseases. Among these are pneumonia at the onset, "bloody bronchitis," cancer, gangrene, abscess, bronchiectasis, tumors, cysts and actinomycosis. (5) Heart disease, especially mitral. As a rule these hemorrhages are slight, yet they may be profuse and may recur for years. (6) Vascular degeneration, the result of increased pulmonary tension, seen in emphysema and arteriosclerosis. (7) In ulcerations of the larynx, trachea, and bronchi, in which cases it may be profuse and rapidly fatal. (8) In aneurisms of the large vessels of the chest it is sometimes sudden and fatal; in other cases the so-called "weeping" may persist for weeks, or the pressure of the aneurism may cause a bleeding erosion of the tracheal mucosa. (9) An extremely rare form of vicarious hemorrhage due to interrupted menstruation. (10) In rheumatism. (11) Malignant fevers, the so-called hemorrhagic type. (12) Purpura hemorrhagica and various other blood diseases, among which are hemophilia, leukemia and scurvy. (13) Distomatosis (Westermanii).

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[62] Amer. Jour. Med. Sci., April, 1902, p. 620.

[63] Johns Hopkins Hosp. Bull., January, 1903.

The amount of the blood expectorated in any of the above conditions may vary from a drop or a few small clots to a quart or more. In general it has a bright red color even when of venous origin since it is aërated in the lungs and is frothy from its admixture with air. The blood may clot in the bronchi and casts of these be expectorated. In gastric hemorrhage the blood is as a rule dark because of the acid gastric juice, not frothy, partly coagulated and is raised by vomiting. If, however, the stomach is empty when a large gastric artery is opened this blood may be bright, while a profuse hemorrhage from a pulmonary artery may be dark and not frothy. Such points would be easy enough to determine were the doctor the observer, but a diagnosis from the history given is often difficult. Paroxysms of severe coughing often cause vomiting, while the coughed blood may be swallowed and vomited, or a little vomited blood may be inspired and set up a paroxysm of coughing. A history of previous lung or stomach trouble is important in diagnosis, also to follow the sputum which for some days after a pulmonary hemorrhage will be blood-tinged, and the stools which will contain blood for a few days after a hemorrhage from the stomach, although during an hemoptysis some of the blood may be swallowed and this also will be found in the stools. It is important to exclude the possibility that the blood in the sputum may have come from varicosities at the back of the tongue or from lesions in the throat, glottis, or esophagus. It is said that the spongy gums of young anemic girls explain the blood in their sputum.

Hemorrhagic Infarction.—Often the diagnosis of a pulmonary infarct may be made from the inspection of the sputum alone. The typical sputum is expectorated in masses which remain discrete in the cup, some of which appear to consist of pure blood, since composed of a very tenacious mucus intimately mixed with fresh blood, while others consist of glairy mucus streaked with blood. After an infarction the expectoration begins suddenly with cough and pain, or, as was true of half of our cases, there is a sudden change in the character of a previous sputum. Microscopically, these masses consist of mucus and red blood-cells. Leucocytes are remarkably few in number, while alveolar cells filled with blood pigment are usually present in enormous numbers. The presence of these epithelial cells, however, may be explained by the fact that pulmonary infarctions are particularly common in mitral valve diseases. In other cases the sputum is much less characteristic, especially if the patient had had considerable sputum previous to the embolism. Sometimes, as in one-third of the Johns Hopkins cases, there followed a real hemorrhage; in other cases the sputum resembles that of pneumonia, while in still others it is more like the brick-red sputum of chronic passive congestion. In one-fifth of these cases the thrombosis was followed by practically no sputum at all. (In one, however, there may have been some blood-streaked sputum before admission to the hospital.)

The amount of blood in the sputum certainly bears no relation to the size of the infarctions. This was well seen in 1 of our cases with very large infarctions and only slightly blood-streaked sputum. All distinctive characteristics of the sputum soon disappear, usually in about 1 week, after which time the sputum is merely blood-stained and soon becomes more watery and free from blood.

Chronic Passive Pulmonary Congestion.—In chronic passive pulmonary congestion, especially that due to mitral valve disease and particularly to mitral stenosis, the sputum is quite characteristic. The patients expectorate, chiefly in the early morning, a sputum which may be uniformly rusty in color but which more often consists of a white mucus background in which are scattered rusty-colored dots or streaks. This rusty color is due to large masses of Hertzfehlerzellen (see page 13). It is in the diagnosis of this condition only that the large number and the constant presence of these cells have much importance.

Malignant Disease of the Lungs.—Patients with tumors of the lungs and bronchi may have no cough and no sputum but more often it is scanty, mucoid or mucopurulent and without distinctive features. An abundant purulent expectoration, sometimes putrid, is to be expected after the tumor masses have undergone necrosis and cavities develop. Blood is present early in the sputum in from a third to a half of the cases, usually in streaks and traces which appear frequently, or it may be so intimately mixed and changed that it gives the sputum a rusty color. Frank and even fatal hemorrhage may occur.

The sputum of cases of cancer of the lung has been described as "characteristically gelatinous, of a red or blackish-red color like currant jelly," but this is by no means common and is seen also in non-malignant diseases. It more often has a prune-juice character and this Stokes thought an important sign. Other patients expectorate a grass-green or an olive-green sputum resembling that of caseous pneumonia. In all cases search should be made in the sputum for fragments of the tumor. These are more likely to be found when the sputum is bloody. Because of secondary infection and necrosis these fragments are usually converted into a conglomerate mass of degenerated cells and debris without characteristic structure. The fresh sputum should be mixed with normal salt solution and examined in a flat glass dish against a black background. All masses should be carefully teased apart and washed free of adherent blood and mucus. Tumor fragments may then appear as reddish, grayish or whitish particles or shreds.

Isolated cancer-cells and cell clusters have been found in the sputum from cases with pulmonary carcinoma. Hampeln[64] regards a sputum composed exclusively or for the most part of unpigmented polymorphous, polygonal cells of variable size, with well-defined nucleus and nucleolus, isolated and in clusters, as distinctive of new growth. It certainly is true, as he suggests, that the normal squamous cells from the surface of the mucous membrane and larynx, also the small polyhedral or cubical cells from the deeper layers of the mucosa of the air passages and the polygonal alveolar epithelium, are found only in rare instances in the sputum, and then only in isolated examples.

Our cases of this condition presented no unusual points. One case with extensive secondary metastasis in the lung had practically no sputum. In another case with a large pulmonary tumor the sputum on 1 day was very viscid, slightly rusty, greenish-red in color, not fetid and consisted of pus, red blood-cells and alveolar epithelium with much myelin degeneration. Later it was of a dirty grayish color, mucopurulent in character and at times contained considerable blood. One case with epithelioma of the bronchus had considerable sputum and several severe hemorrhages, while at other times his sputum was frothy, seropurulent, liquid and blood-streaked. The diagnosis has in several cases been made from tissue fragments found in the sputum.

Patients with mediastinal growths have cough and sputum if the pressure of the tumor produces a bronchitis. If the pressure from the tumor causes a stenosis of a bronchus, a bronchiectatic cavity will result with its profuse fetid expectoration. Gangrene later may develop.

Syphilis of the Lung.—Syphilis of the lung is a difficult problem to discuss with any degree of confidence. Some, especially the röntgenologists, find this condition common; others, especially the pathologists, rare.

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[64] Lord, Diseases of the Bronchi, Lungs and Pleura.

How many of the large groups of pulmonary conditions with extensive scar tissue formation, often with dilated bronchi, and which now are predominately pyogenic infections, were primarily luetic is difficult to say. Funk in McCrae's clinic found 4 cases among 1200 patients who had been thought to be unquestionably tuberculous. Fowler and Godley state: "Evidence of excavation with fetid expectoration which does not contain tubercle bacilli should always suggest the possibility of the case being one of pulmonary lues." The expectoration may be profuse, purulent, and offensive, fetor being a common characteristic in advanced cases. These cases usually pass as advanced tuberculosis with cavity formation although it should in such cases be easy to find Koch's bacillus in the sputum. As a result of stenosis of the bronchi, a common event in this disease and due to the extensive formation of connective tissue at the hilum of the lung, bronchiectatic cavities will form and the sputum present all of the characteristics of this condition. While hemorrhages are not common some cases attract attention by the remarkably bloody nature of the sputum, while some have died of hemorrhage. Some writers state that unless repeated examinations for the tubercle bacilli be made these cases will pass for consumption. Osler, on the other hand, states that he has never seen a case of pulmonary lues which clinically resembled tuberculosis. Fortunately in many cases a positive therapeutic test will clear up the diagnosis, while already in 1 case at least Treponema pallidum has been found in the sputum.

Pneumokoniosis.—According to the dust which is inhaled this condition has received various names: anthracosis, if it is coal-dust; siderosis, if iron-dust; and chalicosis, if it is silicate or other rock-dust. The expectoration is in general mucopurulent, often profuse, and laden with the above-mentioned dusts (see page 4).

Diphtheria.—Cultures should be made from the throat of all patients who are suspected to have or recently to have had diphtheria. Cultures should certainly be made if any membrane is visible in nose or throat, but they should also be made if the throat of one known to have been exposed to diphtheria shows a follicular tonsillitis, or even is merely congested. If a person known to have been exposed has any constitutional symptoms suggesting infection, the bacteriological examination of the throat should be made even though it appears perfectly normal.

Recently a mail carrier, No. 48, admitted July 13, 1914, came complaining of difficulty in swallowing and diplopia. He had not been ill, he had not even been feeling badly. His throat was merely congested but in the nose and nasopharynx was an old slough, in cultures from which Bacillus diphtheriæ grew. It is of interest that there had been an epidemic of diphtheria among the school children along his route.

Examinations should be made at frequent intervals after an attack of diphtheria until at least 2 successive examinations are negative for this bacillus. The nasal secretions also of all persons with chronic coryza who have been exposed to diphtheria should be examined. Cultures should be made from any membrane forming on a superficial wound in the skin or on any mucous membrane. The reason for these careful examinations is not nearly so much for the sake of the patient as for the safety of his neighbors. In addition to these conditions sometimes diphtheria bacilli are unexpectedly encountered.

G. H., No. 9266, aged 7, was admitted for tonsillectomy. There is no history that he had had or had been exposed to diphtheria, but routine examination of the tonsils after they had been removed showed the presence of virulent diphtheria bacilli (tested by inoculation into a guinea-pig). It is of interest that later one of the nurses who had cared for this boy became sick with acute diphtheria.

Cultures and smears are best made from fragments of the membrane (if present) picked from the surface with a pair of forceps. If, however, no membrane is seen material for a culture can be obtained on a swab made up of a wad of cotton wrapped on the end of a stiff wire about 8 inches in length and sterilized in a test-tube. Cultures should not be made from the throat within 2 hours after an antiseptic gargle has been used.

This sterile cotton swab is forcibly rubbed against the edge of the membrane if this is visible and, if not, against any exudate or over any injected area. Smears are made by rubbing the fragment of membrane or the swab on a clear slide, and cultures, by rubbing these forcibly and thoroughly over the moist surface of solidified blood-serum. Failures to get a positive culture are due frequently to the fact that the swab was rubbed over the center of the patch of membrane, and not at the edge, or that the rubbing was not forcible enough, or that the surface of the serum was too dry, or that the swab was not rubbed firmly enough against the serum.

The inoculated serum tube is then put into the thermostat as soon as possible and left there at 37° C. for from 8 to 20 hours. Smears are then made from the growth (for a description of Bacillus diphtheriæ see page 37).

In addition to Bacillus diphtheriæ one often finds in the throat Micrococcus aureus and Streptococcus pyogenes.

The value of the bacteriological examination of the throat is illustrated by the fact that McCollum was able to grow this organism from the throats of 40% of 500 cases whose throat condition clinically suggested, but was not characteristic of, diphtheria, and that in many instances positive cultures were obtained from 24 to 48 hours before any membrane appeared.

Pseudodiphtheria bacilli resemble Bacillus diphtheriæ, and yet their morphology is not quite the same, since they do not show bipolar staining and are often shorter and a little thicker than the typical form. They differ also in their cultural characteristics and much in their pathogenicity. This group certainly includes the pseudodiphtheria bacillus of Hoffmann, the xerosis bacillus and others.

For the bacteriologist this problem is interesting and important, but for the clinical laboratory worker this classification has but academic interest. He should work only with fresh smears and with cultures less than 24 hours old. If using such material he calls Bacillus diphtheriæ all organisms whose morphology is characteristic, he will make fewer mistakes than 1 in 100, and this 1 case will not be hurt if treated for diphtheria, while the community will be safer because no chances were taken.

Vincent's angina, "*Plaut's angina,*" "*ulceromembranous stomatitis,*" and "*ulceromembranous angina,*" are some of the names applied to acute or subacute febrile infections of the tonsils or mouth, characterized by the formation of deep penetrating ulcers often covered by a pseudomembrane, in which large numbers of certain bacteria can be demonstrated.

To demonstrate these organisms the base of the ulcer is mopped with a sterile cotton swab and smears at once made, dried, run through the flame 3 times and stained with carbolfuchsin.

In typical specimens the field will be found crowded with various cocci and bacilli and especially with Bacillus fusiformis and a spirocheta.

It is very important that the student study the flora found in the mouth. The number of organisms usually found there is great, over 100 different forms having been described. Among these there are some so constant that they are considered the natural mouth-flora: bacilli, spirilla, and various leptothrix and spirocheta forms, many of them huge, many showing grotesque involution forms, but all with one common characteristic, that they are very hard to cultivate. Among these are Bacillus fusiformis and Spirocheta dentalis (Miller). Whether any of these are pathogenic or not is a question, but one thing is certain, that they increase in great numbers in any ulcerative process in the mouth and probably do aid in the formation of the ulcers. Undoubtedly they aid in the decomposition of the exudate of these ulcers, and explain much of its bad odor. It is very likely that Bacillus fusiformis is important in the production of Vincent's angina, but the problem is a difficult one, for smears from ill-kept mouths without ulcers show such remarkable pictures that the smear from the base of an ulcer must be very rich indeed in long bacilli and spirochetæ before some will grant it any importance whatever in diagnosis.

CHAPTER II

THE URINE

GENERAL CHARACTERISTICS

The Collection and Preservation of Urine.—It is important in all quantitative chemical work which involves the urine that a complete and well-mixed 24-hour specimen be collected. In many hospitals the day's collection begins at about 6 A.M. The patient voids at this hour and that specimen is discarded. All the urine is then collected until 6 A.M. the next day, at which time the patient voids, which specimen completes the collection. In case one plans to separate the day urine and the night urine, the former period would extend from 6 A.M. to 9 P.M. and the night from 9 P.M. to 6 A.M., the hours during which the patient is, as a rule, asleep. One then estimates the elimination per hour.

It is necessary that the specimen be collected in a clean bottle and that some means be used to prevent the growth of bacteria which under ordinary conditions is very rapid. If no refrigerator is set aside for this purpose a chemical preservative is necessary; which, will depend on the tests to be made. If we have chemical work in view we usually use chloroform, enough so that at least 1 drop remains undissolved at the bottom. The bottle must be tightly corked or bacteria certainly will grow in the upper layers of the urine from which the chloroform is volatilizing. This reagent adds nothing to the volume of the urine and can be entirely removed. Chloroform does not preserve the formed elements, yet it is so satisfactory for chemistry that the content of oxybutyric acid in the urine will remain unchanged for years. A few crystals of thymol or of gum camphor are often used. Formalin is valuable since it preserves the formed sediment of the urine. It has, however, two disadvantages; it is an active reducing body itself and it adds a sediment of its own. A slight objection to thymol is that the urine will give a test suggesting bile. Others add to the urine one-fifth its volume of dilute chloroform water or of saturated borax solution. The specimens, however preserved, should if possible be kept in an ice box.

Sometimes a 24-hour specimen is not desired. For instance, in the diagnosis of slight chronic nephritis a comparison of the urine voided first in the morning and of that voided at the end of a day's work gives valuable information, or we may ask the patient to exercise violently and examine the next voiding. Again, in a mild case of diabetes mellitus only that urine voided 3 or 4 hours after a hearty carbohydrate meal may contain sugar, and if this voiding were mixed with the entire 24-hour specimen the sugar might be in too dilute solution to be detected. For microscopical examination the urine should be studied as early as possible after it is voided, and, if possible, without the addition of any preservative.

The value of the examination of the urine as a routine practice needs no emphasis. The perfectly healthy appearance of the patient is no guarantee that the urine will not clear up the diagnosis. The surgeons especially need this warning, for all too often 1 urine examination would probably have prevented an operation following which the patients have died in diabetic coma.

The Amount of Urine.—The limits of the daily amount of urine to be considered normal vary widely. Generally speaking we say that an adult should not void less than 800 c.c. or more than 3000 c.c. per 24 hours. The average output is usually stated as from 1500 to 2000 c.c. That may be true for a country in which beer drinking is common, but in this country from 900 to 1500 are given as normal limits. The average daily output of women is slightly less than of men. The amount of urine depends in part on the size of the person. That of an adult is almost directly proportional to his weight, but children excrete relatively more than do adults; newly born infants void from 150 to 200 c.c. a day, and children from 3 to 5 years of age about 700 c.c.

The amount of urine per day in a normal person depends chiefly on the volume of fluids ingested. By varying his fluid intake this may vary from 800 to 3000 c.c. per 24 hours. The increase in the output after drinking a large amount of water at one time reaches its maximum in from 2 to 3 hours and lasts from 5 to 6 hours. Yet, as all have experienced, the water output is as capricious as is that of the other urinary constituents.

The margin of functional ability of the kidney is surprisingly large, as is seen in diabetes mellitus, in which disease a practically normal kidney may eliminate each day 25 liters of water, an absolutely increased amount of the normal solids and several hundred grammes of sugar and will endure this increased work for a long time without showing any sign of disease. In the case of a rabbit Kulz was able by intravenous injection of salt solution to increase the output of urine to 256 c.c. per hour for 9 hours, and yet the qualitative composition of this urine remained normal. Insensible as well as copious perspiration affects the amount of urine, and so, other things being equal, it will be greater in cool weather than in hot. The amount voided in health as well as in disease is also affected by the loss of fluid in other ways, particularly by diarrhea and by vomiting.

The water content of exudates (pleural or ascitic), of subcutaneous edema and of other abnormal accumulations in the body is finally excreted through the urine. This explains the polyuria seen in nephritis while the edema is disappearing. To demonstrate this the person should be put on constant diet and constant fluid intake and the urine carefully measured or the increased output may pass unnoticed.

The differences in the hourly amounts of urine voided during the day and during the night has not received the attention it deserves. Quincke and his students found that in liver, kidney, and in heart diseases which produce edema the urine voided per hour during the night is greater in amount and contained more solids than that during the day, a condition sometimes called nycturia. Normally the reverse is true; the kidneys seem to sleep with the rest of the body and secrete an amount per hour during the day which is to that per hour during sleep as 100 : 50 to 60 or perhaps as 100 : 80 to 90. In cases of cardiac or arterial disease and in nephritis the reverse is true. This is called " the fixation of specific gravity." It would seem in such cases as though the kidney improved its opportunity during the sleeping hours to eliminate that which it could not during the day. In a well-marked case of nephritis, D : N : : 100 : 200 and in 1 case [1] which we followed the ratio was even 100 : 544. This does not depend, we are convinced, on changes in the position of the patient's body and therefore on changes in circulation depending on this. This test has definite diagnostic value since it aids to differentiate cases of functional circulatory and renal disturbance (*e.g.*, hysterical) from organic diseases. The disturbance of this ratio is particularly marked in case the output of urine is increased, as in diabetes, or by diuretics or by exercise during the day. The disturbed ratio is not present in heart disease providing the compensation is good. Cardiac insufficiency seems the underlying cause in most cases of the disturbed ratio.[2]

By *polyuria* is meant an output of over 3000 c.c. of urine in 24 hours. An output of 800 c.c. or less in 24 hours is termed an oliguria. To be important clinically an increase or diminution in the output of urine should extend over several consecutive days. These limits are quite elastic, even for normal persons.

Pathological Factors Influencing the Amount of Urine.—(1) The condition of the renal parenchyma in diseases with bilateral diffuse lesions. A general rule is that the more acute the nephritis the less the amount of urine excreted. In acute nephritis there may at first be anuria, or 50 to 100 c.c. only per day; in a subacute nephritis about a normal amount, while in a chronic interstitial nephritis the patient may void from 6 to even 12 liters each 24 hours.

(2) The velocity of the blood-current through the kidney determines in great degree the amount of urine, the general law being that the amount of urine varies directly as the amount of blood which passes through the kidney in a unit of time; that is, as the rapidity of the blood-flow, and not as the blood-pressure. Hence chronic passive congestion of the renal circulation, whatever the cause, diminishes the output and drugs which improve the renal circulation increase the output and so act as " diuretics."

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[1] Johns Hopkins Hosp. Rep., vol. x, p. 323.

[2] Laspeyres, Deut. Arch. f. klin. Med., August 16, 1900.

(3) *Disturbed Metabolism.*—The output of urine depends much on the quality and the quantity of the substances to be eliminated. The best illustration of this is the patient with diabetes mellitus who may void even 2·5 liters of urine per day when his sugar output is high and when, by modifying his diet, the sugar output is reduced, the water output diminishes as well. The so-called "epicritical polyuria" which occurs after fevers may have a similar explanation although we do not know the substances involved. Some cases of typhoid fever, for example, during convalescence void from 4 to 6 liters of urine per day, and the same may be true of almost any disease which has diminished the output of urine. This is beautifully seen in cases of subacute parenchymatous nephritis. In such cases a polyuria accompanies the elimination of substances which were retained during the acute period of the illness and indicates a favorable prognosis.

(4) Psychical disturbances and various nervous storms may be followed by polyuria, as may also angina pectoris, hysteria, and epileptic convulsions. The cause is probably vasomotor. The so-called "paroxysmal polyuria" is probably a functional disturbance.

(5) Another cause of periodic polyuria is the hydronephrosis seen in movable kidney, etc.

(6) Chilling of the skin may be followed by polyuria.

(7) Of the various forms of polyuria the causes of which are unknown, diabetes insipidus is the most striking example. A patient with this disease may void even 12 or more liters in a day.

(8) Hydremia.

(9) Lesions of medulla.

(10) Stimulation of renal secretion by drugs or "renal diuresis" due to the action of *e.g.*, the caffein group.

It is often of interest to estimate the proportion of water intake which the urine represents. The normal person eliminates from 60 to 70% of water intake through the kidneys. If he increases greatly the amount of water consumed the bulk of the increase will appear in the urine and the percentage of urine relative to total intake may rise to even 96%. In 2 cases of chronic interstitial nephritis however the output of water through the kidneys was relatively high even though absolutely small. One of these cases with an intake of 1960 c.c. voided 85% and on the next day, of the 2400 c.c. he drank, he eliminated 86% through the kidneys. In another case, of 1370 c.c. of fluid ingested, 85% was voided in the urine and on another day 83% of 1790 c.c. In chronic parenchymatous nephritis, with the patient in almost stable condition and receiving exactly the same amount of fluid each day for 26 days (6200 c.c.), the average daily output by the kidneys was 66%. In cases with ascites and signs of renal insufficiency the renal output will drop to 40% of the intake and in anuria even to 0. The following figures from a recent case of eclampsia in the obstetrical ward illustrates this well. The patient was not urged to drink much. On the first day after the convulsions, of 8350 c.c. of water drunk the kidneys excreted 20%; the next day, of 10,535 c.c., 80%; on the fourth day of 9400 c.c., 93%; and on the fifth day the patient drank 7100 c.c. of water and voided 7390 c.c. of urine. During this time there was some diarrhea.

Anuria, or the absence of micturition, whether from failure of renal secretion or from retention of the urine, may be due to a variety of causes, which may be classified as obstructive, paralytic, septic, renal, and prerenal.

The best illustration of the obstructive type of anuria is that seen in tumor or hypertrophy of the prostate gland, in vesical calculus, or in trauma of the urethra. The paralytic form follows transverse lesions of the spinal cord, but the case which develops early and insidiously in typhoid fever deserves particular mention since it is unfortunately so often overlooked. The reflex type is best illustrated by cases with a calculus in the pelvis or ureter of 1 kidney, or an operation, *e.g.*, nephrectomy of 1 kidney and reflex inhibition of the secretion of the other.

The most important form of anuria is that due to renal disease. In all cases of acute nephritis there is a reduction of the urinary output which varies in direct proportion to the intensity of the acute process. In such cases usage has permitted the term anuria to include cases who void a little but negligible quantity (*e.g.*, 100 c.c. or less) in 24 hours.

The prerenal causes are numerous. This form of anuria may be due to purely functional causes, as hysteria, in which case it is temporary and is followed by a polyuria; or to certain fevers, as scarlet fever; to certain poisons, as phosphorus, lead, turpentine, ether and chloroform; it occurs in collapse; and often, but not always, with approaching death.[3] The anuria of Asiatic cholera is attributed to inspissation of the blood. It is surprising how long a person can live after all renal tissue has been removed or destroyed or when there is complete suppression of renal function. Moxon reported a case of ureteral calculus with anuria lasting 14 days, which recovered after the passage of the stone. Adams' patient had anuria for 19 days and yet recovered. Polk's patient lived for 11 days after his 1 and only kidney had been removed.

Specific Gravity.—The specific gravity of the urine is its weight compared with that of an equal volume of water. The standard is the weight of a liter of water, 1000 gms. (some say, of a cubic centimeter of water, 1.0 gms.). If urine has a specific gravity of 1018 (or 1.018) we mean that a liter of it would weigh 1018 gms. (and 1 c.c., 1.018 gms.). The specific gravity of any fluid may be determined accurately by weighing a given amount of it in a pycnometer, but clinically it is determined by measuring its buoyancy by means of a form of aërometer called a urometer. The aërometer spindles are usually graduated from 1000 to 1050. It is better to use 2, 1 graduated from 1000 to 1020, the other from 1020 to 1040. The practitioner should be careful to get good instruments, for some on the market are very inaccurate, especially those of the smaller types. The glass cylinder to be used should have parallel sides, fluted if possible, a wide base and a good spout. This is filled about ⅘ full of urine and the foam, if present, removed with a piece of filter paper. The bobbin is then dropped in and allowed to come to rest. The observer should now assure himself that it actually floats and does not touch the side of the cylinder.

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[3] See also Bevan, Am. Surg., April, 1903.

While making a reading the eye should be on the level of the base of the meniscus. Two or 3 separate readings should be made, the bobbin being pushed down each time and then allowed to come to rest. It is very important to make the proper corrections for temperature. These instruments are standardized usually for 15° C. A difference in temperature of 3° C. means a difference of 1 in the fourth place of the specific gravity reading. That is, a urine which at 15° C. has a specific gravity of 1.012, will at 18° C. read 1.011. This correction is usually of slight importance if the urines are of average concentration, yet we suspect that failures to consider it explain the impossibly low figures of the specific gravity of the urine of certain cases of diabetes insipidus and chronic interstitial nephritis. This correction must of course be carefully determined if the specific gravity is to be used as the basis of quantitative work, as for instance, the estimation of the total solids or the amount of sugar or of albumin. It is only fair to say that for the latter we think the aërometrical methods at their best are hardly accurate enough, and that the urine should be weighed on a good chemical balance. Again, an instrument suited for salt solutions is not always accurate in a sugar or albumin solution.

In recording the specific gravity of urine mention should always be made of the character of the specimen examined, when voided, etc. In general, only a well mixed 24-hour specimen should be tested, for the specific gravity of the various voidings during the day and night may vary from 1.002 to 1.040, depending on the food, the fluid, the lungs, the skin, etc. It may be very high after severe exercise with sweating, after transudate formation, etc. Two normal men recently were refused on first examination by life insurance companies because they happened to have eaten food just before examination which for them had a diuretic action and so the specific gravity of the urine was abnormally low, in 1 case 1.003.

The specific gravity of the mixed 24-hour urine of the normal adult varies from 1.015 to 1.020. In the new-born from 1.005 to 1.007.

In case the amount of urine is too small to fill the cylinder, it may be diluted to a known volume. The formula for the correction is: Sp. gr. = 1.000+ab, in which "b" = the dilution, and "a" the last 2 figures of the specific gravity found. For instance, if the urine was diluted with just twice its volume of water and if the reading of the diluted urine was 1.006, Sp. gr. = $1.000+3\times6=1.018$.

In some cases it is the specific gravity of the single voidings which are desirable. For instance, in the diagnosis of an early chronic diffuse nephritis the constantly low specific gravity of the morning urine is of value (see page 312).

The specific gravity of the urine depends chiefly on the relative amounts of water, urea and sodium chloride which it contains. The amount of water will depend on the factors discussed on page 90. A high percentage of urea explains in large measure the high specific gravity of the urine in fevers. The amount of salts is increased by foods, by the medicines taken and by the absorption of transudates. While in general the specific gravity of the urines of any 1 person will vary inversely as its amount, this is not strictly true since an increased output of water increases the output of solids. A marked exception to the rule is diabetes mellitus, in which disease the urine is greatly increased in amount and also in specific gravity; while another exception and in the opposite direction is seen in nephritis with renal insufficiency, in which case there is oliguria and a greater diminution in the output of solids than of water and therefore a low specific gravity. In nephritis a definitely low specific gravity is rather suggestive of an impending uremia. It is also seen in cases of malnutrition in which the metabolic processes are at low ebb, as for instance, in a patient of Chabrié, a girl of 20 years of age, whose output of urine on 1 day was but 750 c.c. with a specific gravity of 1.008. In diabetes insipidus the specific gravity is very low (some ridiculous figures are probably explained by the failure to make a correction for temperature).

An approximate estimation of the total amount of solids in the urine may be made by the use of *Häser's coefficient*, 2.33. The last 2 figures of the specific gravity multiplied by this empirical coefficient will give fairly accurately the number of grammes per liter of solids excreted.

Authorities disagree concerning this coefficient. Neubauer gives 2.328. Donze[4] states that the coefficient should be slightly lower for dilute than for more concentrated urines and therefore should vary from 1.850 to 2.440, with an average of 2.210.

The specific gravity of the urine may be used in the quantitative estimation of sugar, albumin, etc. If used for this purpose, however, a good urometer should be used and the temperature correction carefully made, otherwise the result may be absurd.

Color.—The color of the urine of a normal adult is usually a shade of yellow, its depth varying with the dilution of the urine and hence directly with its specific gravity, a dilute urine being of pale and a scanty urine of dark yellow color. Exceptions to this are: diabetes mellitus, in which case the urine is very pale and yet is increased in amount and has a high specific gravity, a point which will sometimes suggest the diagnosis; in aplastic anemias, especially chlorosis, in which cases the urine is pale from lack of pigment, while in those anemias in which there is increased destruction of the red corpuscles, as in pernicious anemia, the urine is highly colored. As a rule acid urine is more highly colored than is alkaline urine. In uremia the urine often is very pale, a fact which was responsible for the old theory that a retained pigment is the cause of this condition. In cases of certain grave infections which seem to have destroyed the bile-producing function of the liver the urine is said to have been without any pigment at all. A febrile urine is dark since it is concentrated and also because it contains more of uroerythrin and other pigments than does normal urine. Urine turns dark on being exposed to sunlight. For this reason the contrast in color between the day and night urine is often striking, the day urine being of a golden-yellow and the night of a pale green color. This difference is due partly to the amount of pigment excreted but more to the effect of sunlight which changes the colorless chromogens to pigments in the specimen collected during the day.

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[4] Compt. rend. Soc. de Biol., 1903, 155, 537.

The use of a color scale is recommended in order to avoid the variety of terms used to describe the same color (yellow, light yellow, amber, orange, straw, etc.).

The pigments normally present in the urine are:

UROCHROME, the predominant one, gives urine its normal shade of yellow, orange or brown, according to the amount present. It has not yet been isolated and so its empirical formula is not yet known. Indeed several pigments may be included under this name. It has no absorption spectrum and no fluorescence. There is evidence that it is derived from urobilin.

HEMATOPORPHYRIN in small amounts is present in every normal urine (see pages 97 and 242).

UROERYTHRIN is often present under normal conditions and explains the salmon-red color of the urate sediment. It is increased in amount by a rich meat diet, by profuse sweating, alcoholic drinks, violent exercise and by certain digestive disturbances. It is increased also in fevers, in circulatory disturbances and in arthritis. This pigment may be demonstrated by shaking the urine out gently with amyl alcohol, which will become orange in color and will give the characteristic spectrum of uroerythrin. This pigment bleaches in a characteristic manner on exposure to light. When dissolved in concentrated sulphuric acid its solutions are carmine-red, which on the addition of an alkali changes to purple, then to blue and finally to green.

Urobilin is a constituent of the normal urine, in amounts varying from 30 to 120 mg. per day. Urobilin itself is not present in perfectly fresh urine, but its chromogen, urobilinogen, is, and this on exposure to sunlight yields urobilin. In the following lines we shall include under the term " urobilin " this pigment and its chromogen or chromogens.

Whether urobilin is a single pigment or a group of pigments is a doubtful question. It has been impossible to isolate it without some decomposition and all efforts to remove its impurities have thus far failed.

The origin of the urobilin present in normal urine is still in doubt, but evidence favors the theory that it originates in the intestines and in the liver. There is no constant relation between urobilinuria and urobilinemia, or between bilirubinemia and urobilinuria. The attractive theory of Gilbert and Herscher and others [5] that the kidneys transform the bilirubin of the blood into the more diffusible urobilin has not received confirmation. Conner and Roper [6] found that bilirubinemia and urobilinuria bear a rough quantitative relation to each other and yet their work furnishes little support to the theory that the urobilin of the urine originates in the kidneys. Certainly a great deal of urobilin is formed in the intestine (enterogenous formation) as the result of the reducing action of certain bacteria on bile pigment. It seems to be identical with stercobilin.

Some urobilin is said to be formed in areas of hemorrhage into the body tissues (histogenous formation), and, indeed, wherever there is blood destruction from any cause (hematogenous formation) as after toxic doses of blood-poisons, such as antifebrin and antipyrin. Meinel [7] found that a certain amount is formed in the stomach in some cases of hyperacidity.

The urobilin of the urine is increased also in fevers, in chronic passive congestion, lead poisoning, atrophic cirrhosis of the liver, etc. It is increased before and after a period of obstructive jaundice. There is a life-long increase in persons with chronic family jaundice (Tileston and Griffin).

When there is a marked urobilinemia there may be also a definite urobilin jaundice.

Urobilin does not give Gmelin's test; it gives a test similar to the biuret; and if to a urine made strongly alkaline with ammonia and filtered be added a 1% alcoholic solution of zinc chloride the presence of urobilin will be indicated by a beautiful green fluorescence and spectroscopically by the characteristic spectrum of alkaline urobilin. The spectrum of acid urobilin may be obtained with urine to which have been added a few drops of a mineral acid, but it is better to shake it out with amyl alcohol and then examine the extract, or to add an equal amount of 10% ZnAc in absolute alcohol, and filter.[8] This test will be given even in spite of the presence of considerable bilirubin. The fluorescence is best seen with a convex lens which gives a luminous green circle.

For the quantitative determination of urobilin Hoppe-Seyler recommended to acidulate 100 c.c. of urine with sulphuric acid, saturate it with ammonium sulphate and allow it to stand for some time; then filter and wash the precipitate with saturated ammonium sulphate solution. The precipitate is then pressed out between blotting papers and extracted repeatedly with equal parts of alcohol and chloroform. The extract is then filtered into a separating funnel and to the filtrate are added 2 volumes of water and then chloroform until the chloroform settles out in a clear layer. The chloroform solution is evaporated on a water-bath and the residue dried at 100° C. It is then extracted with ether, the ether extract filtered off, the residue dissolved on the paper in alcohol, again brought into the weighed beaker, evaporated, dried and weighed.

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[5] Compt. rend. Soc. de Biol., 54, p. 795.
[6] Arch. Int. Med., Jan., 1909, vol. ii, p. 532.
[7] Centralbl. f. inn. Med., 1903, vol. xxiv, p. 321.
[8] Schlesinger, Deutsch. med. Wochenschr., 1903, No. 32, p. 561.

Among other chromogens in the normal urine are indoxyl-sulphuric acid (see page 143), indoxyl-glycuronic acids and possibly skatoxyl-sulphuric and skatoxyl-glycuronic acids. Among the pigments which may under pathological conditions be present are hemoglobin, methemoglobin, hematin, bile pigments, melanin and others; some come from drugs, *e.g.*, chrysophanic acid; others from the foods, *e.g.*, the pigments of various berries, cherries, etc.

Blood.—The color of a urine which contains blood will depend on the amount of blood present and on the modifications which the blood-pigment has undergone: hemoglobin gives the urine a reddish tint, methemoglobin a brownish one. When only a little blood is present the urine often has a characteristic smoky tint due to methemoglobin. When more is present its color may be reddish-brown, brown, almost black, or greenish-black, as in the black-water fever of hemoglobinuria. Such urine is cloudy because of the large number of blood corpuscles and other organized elements of sediment usually present. If the sediment is very heavy one may find in it masses of amorphous hemoglobin.

Hematoporphyrin.—Hematoporphyrin may be present in the urine in large amounts after the long continued use of trional, sulphonal, or tetronal; also in cases of typhoid fever. Thick layers of such urine have a dark or blackish color; thin layers, a yellowish-red or violet color. The black color sometimes seen in such cases is, Garrod thinks, due only in part to hematoporphyrin and more to some unstable purple pigment.

Bile.—The urine of the jaundiced patient usually contains bile pigment, but in cases of very mild jaundice urobilin alone may be present. The color of the urine which contains bilirubin and biliverdin may be dark yellow, brown, green, greenish-black, or in long-standing cases even quite black, depending on the amount of bilirubin which has become changed to biliverdin and other modifications of this pigment. If an acid urine which contains considerable bilirubin be allowed to stand in a cold room a sediment of bilirubin in needle crystals may be deposited. It is often possible to detect the presence of even very small amounts of bile in the urine by shaking this enough to produce a foam. This foam, white in all other urines no matter how dark they may be, is stained yellow by bile; it may also be yellow if very much urobilin is present.

Melanin is present in the urine in cases of melanotic tumors which have invaded the viscera, especially the liver (Garrod). Such urine may be black but more often is quite normal in color when voided, since the pigment is then present as the colorless chromogen, which later is split by sunlight, yielding melanin. The change in color begins at the top and extends downward through the urine forming sometimes a sharply defined black layer above one of colorless urine. This transformation of melanogen may be hastened by the addition to the urine of nitric acid or of any other oxidizing body, especially ferric chloride, which will at once turn the urine black and throw down a gray precipitate which is soluble in excess of this reagent. This, the ferric chloride test, is the most delicate and reliable of all tests of melanin and necessary for its recognition.

HOMOGENTISINIC ACID, the chief pigment present in the condition named "alkaptonuria," gives to the clear urine after standing or after the addition of an alkali (see page 208) a brownish-black color and a syrupy consistency.

The urine is sometimes very dark, almost black, in cases of peritonitis, gangrene of any organ, and in any condition, including simple constipation, which favor the formation of the aromatic products of decomposition, the ethereal sulphates of indoxyl, etc. In these cases the urine is sometimes very blue, not from indigo but from higher oxidation products of indol. Such urines, clear at first, will blacken if nitric acid be added and they are then warmed. They do not blacken on the addition of ferric chloride and do not reduce copper solutions.

Some urines are very dark when voiding, others only when they have stood a long time. This color may be due to pyrocatechin, $C_6H_4(OH)_2(1.2)$, which in alkaline solution is oxidized by the air to a greenish-brown and finally black color. Such urine will reduce alkaline copper sulphate if heated but will not bismuth. Some (*e.g.*, Baumann) believe that pyrocatechin is derived from the vegetables of the food.

To isolate pyrocatechin the urine is concentrated by heat, then filtered, a little sulphuric acid added, and then boiled to drive off the phenol. It is then shaken out repeatedly with ether; the ether is distilled off, the residue neutralized with barium carbonate and again shaken out with ether. The ether is then allowed to evaporate and the pyrocatechin will crystallize out.

HYDROCHINON, $C_6H_4(OH)_2(1.4)$, is present in the urine after the use of phenol. Its decomposition products give to the urine a dark color and reduce copper solutions easily.

Urine containing INDOXYL in large amounts is clear when voided, but soon becomes dark from the presence of indigo. The blue of the indigo may be masked by the yellow color of the urine. The scum of such a urine may be blue. Sahli mentions such a case, that of a boy whose urine when voided was of a green-grass color.

Ochronosis is a rare disease characterized by blackening of the cartilages. The urines of these patients turn black on standing. Osler reported 2 such cases whose urine contained the alkapton bodies, but it is said that in other cases the black color of the urine is due to other pigments.

Garrod [9] classifies the causes of *black and very dark urines* as follows: long-standing jaundice; hematuria or hemoglobinuria; melanotic sarcoma; alkaptonuria; ochronosis; indoxylsulphate in great abundance; certain cases of tuberculosis in which cases the urine must stand for some time, even a month before the color develops; certain drugs, as phenol; and rare cases due to unknown pigments. Of these cases in only 2 is there really black urine: melaturia, and alkaptonuria on standing.

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[9] The Practitioner, 1904, vol. lxxii, p. 383.

In CHYLURIA the urine has a milky appearance.

COLORS DUE TO MEDICINES.—The list of medicines which may modify the color of the urine is too long to tabulate. In general it may be said that if the urine of a patient has an unusual color inquiry should always be made concerning the previous medication. Among the drugs which deserve mention are carbolic acid, whether applied internally or externally (in which case the color of the urine is important to control therapy), tar preparations, resorcin, naphthol, salol, and many aromatic bodies. The change of color may appear only if the urine is alkaline and has stood for a long time. Methylene blue even in small amounts, *e.g.*, 0.1 gm., will color the urine for several days. In 1 hour after the dose the urine has a greenish color, later a deeper green, then a blue, which may last even 3 or 4 days. This color may be intermittently present, *e.g.*, only in the first morning voiding. It may be intensified or even be made apparent by boiling the urine after adding acetic acid since the pigment is in part voided in colorless form. Weber[10] thinks methylene blue explains practically all the blue and green urines and doubts that any are due to indigo blue. He emphasizes the common use of methylene blue to color candies and food-stuffs.

Drugs containing chrysophanic acid, *e.g.*, chrysarobin, rhubarb, santonin, senna, and others, give the urine a yellow tint when acid and a red tint when alkaline. The pigments of many vegetables and fruits will change the color of urine, *e.g.*, turnips, whortleberries, blackberries, etc.

Odor.—The odor of normal fresh urine is not unpleasant. The unpleasant so-called "urinary" odor is due to the ammoniacal decomposition of urea by bacteria. That of a decomposing albuminous urine may be especially disagreeable. The urine of patients with cancer of the bladder and deep inflammatory disease of the urinary tract may have an intolerable odor. Chabrié believes that the urine has a characteristic odor in certain abnormalities of metabolism, such as diabetes and oxalura. We may even imagine from his writings that he thinks that one of the great masters of French medicine could diagnose insanity from the odor of the urine alone. The urine is said to have a special odor in chyluria and even in hematuria. Sometimes the urine has a remarkable absence of odor. We have noticed a strong odor of H_2S in the quite fresh urine of certain nephritics. It should always be remembered, however, that the bottle in which the patient brings the specimen may explain the odor.

Certain odorous substances are excreted as such in the urine, *e.g.*, valerian, asafetida, coffee, and various foods. Others build odorous bodies, *e.g.*, balsams, copaiba, cubebs, etc. Turpentine gives the urine the odor of violets; asparagus that of methyl-mercaptan.

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[10] Lancet, September 21, 1901.

General Appearance.—The fresh urine of a normal person is quite clear. The one exception is the so-called phosphaturia (see page 101). A faint cloud, named the nubecula, soon appears in the upper layers of a clear urine, which consists of mucous strands enclosing a few cells. After standing, any normal urine may become cloudy; if acid, from a urate sediment (see page 245), if alkaline because of the rapid growth of bacteria which form ammonia from the urea, from a precipitate of the phosphates. In pathological urines a cloud when the urine is perfectly fresh may be due to bacteria, to precipitated phosphates or to an abundant organized sediment.

Reaction.—The fresh urine of a normal person is acid or amphoteric; in certain cases of phosphaturia it is alkaline. The quantitative determination of the reaction of the urine has proved a very attractive field but the results are far from satisfactory. Until recently the "degree of acidity" of a solution was understood to mean the amount of hydrogen which could be replaced by the metal of an alkaline solution (NaOH) regardless of the previous state of the hydrogen, whether free as hydrogen ions or in combinations which could be easily disassociated and the hydrogen substituted for by the metal of the alkali.

The physical chemists define "degree of acidity" as the absolute number of disassociated H-ions in each liter of urine. Judged by this standard urine is only about 30 times as acid as is distilled water and only about $\frac{1}{10}$ as acid as titration would indicate. Is the determination of either of these "degrees of acidity" of value and if so, which? One great difficulty is that that acidity determined by titration is due to a considerable number of chemical substances, the most of them acid salts, and hence the question of color indicator is a very serious one, since the points indicated by the various ones as the neutral point differ much. Phenolphthalein is the indicator usually used. This has as practical advantage the sharpness of its end reaction and the fact that of the indicators it itself is the weakest acid. But it is a poor indicator, perhaps the worst, in the presence of ammonium salts. Whatever results are obtained with it have not an absolute but an empirical value. In the case of man the reaction of the well preserved and well mixed 24-hour specimen of urine is always faintly acid to litmus to a degree corresponding to about 1.15 to 2.3 gms. of HCl for each 24 hours. This acidity depends chiefly upon the diet and is greater the more the proteid ingested. The urine of herbivorous animals is alkaline since the organic acids of their food are oxidized to alkaline carbonates. Yet if these animals are starved their urine will be acid to litmus, since their tissue proteid then becomes their chief food. The urine of a man on a vegetable diet will be less acid, or even amphoteric, from the increased ingestion of alkali-forming foods.

In no case is there any free acid in normal urine, but rather acid salts, especially diacid sodium phosphate, and many others produced by the oxidation of neutral proteids. Among these are salts of sulphuric, phosphoric, hippuric, oxalic, and the oxyaromatic acids. Just what part each of these plays in the acidity of the urine, however, cannot be decided. Certainly uric acid is not a factor, since its solution is neutral to litmus.

The urine of a starving man may have an acidity of constant value, but that of others shows constant variations due especially to the diet and known as the "alkaline tide." The acidity is highest in the morning before breakfast and lower for a few hours after each meal, especially after the breakfast, due to the secretion of the hydrochloric acid of the gastric juice. The acidity is later restored to its previous value when the hydrochloric acid is reabsorbed.

For a short time after a meal, from 2 to 4 hours, the urine may even be alkaline when freshly voided and hence turbid with a sediment of phosphates of the alkaline earths.

Phosphaturia is the name given to an interesting symptom-complex characterized by the presence of a heavy precipitate of the earthy phosphates in the freshly voided urine. Formerly, as the name would imply, this was supposed to be due to an increased output of phosphoric acid. There is, however, no such increase and the precipitation is due rather to a change of reaction, for the phosphates can remain in solution only in acid medium, so that the name "alkalinuria" would be much more suitable. Phosphaturia (*i.e.*, alkalinuria) may be present if the diet consists of vegetables; in cases of gastric diseases with considerable loss of hydrochloric acid to the body through vomiting or lavage and perhaps through diarrhea also; and in a group of nervous patients without either of the above mentioned causes. In this last mentioned group the phosphoric acid output during the periods of "neurasthenia" with phosphaturia has been found diminished to about half its normal value, the nitrogen output decreased, but the calcium output increased. The trouble would seem to be an excess of the output of calcium relative to that of phosphoric acid. In Soetbeer and Krieger's case the phosphoric acid output was practically normal, the calcium was increased even to 0.7 gm. a day (normal 0.2) and $Ca : P_2O_5 : : 1 : 1.5$ to (normally $1 : 12$). Some cases[11] would seem to have during their periods of phosphaturia symptoms definitely referable to or coincident with this abnormal metabolism, but due to changes in calcium metabolism rather than to that of the phosphoric acid. In 1 case the calcium output was increased more than 3-fold, perhaps as a result of chronic colitis. Phosphaturia occurs also after sexual excesses and during the periods of depression following psychical exaltation. Freudenberg[12] carried this idea to an extreme, separating phosphaturia, latent phosphaturia (in which the phosphate precipitate appears when the fresh urine is heated), and ammonuria (in which case moist litmus held over the mouth of a tube of heated urine will turn blue). He thinks that these are 3 grades of the same abnormality, which is found in sexual neurasthenics especially but not in patients with true hysteria. It is often met with among mental cases (Heinicke).

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[11] Soetbeer and Krieger, Deutch. Arch. f. klin. Med., 1902, vol. lxii, p. 553.

[12] Deutsch. med. Wochenschr., September 17, 1903.

There are a few cases with general neurasthenic symptoms in whose urine the phosphoric acid is definitely increased, and who later perhaps will develop polyuria or glycosuria. Senator suggests that some of the cases of diabetes insipidus, the specific gravity of whose urine is higher than in other cases, may belong here.

The reaction of the urine can be much modified, even made alkaline, by drugs, particularly by large doses of the alkaline salts. Milk of lime in sufficient doses will make the urine alkaline from the presence of ammonium carbamate (Abel). While a transudate is being absorbed the urine may be alkaline, also after a hemorrhage into the intestine, in which case it is due to the absorption of the blood-salts. The urine is alkaline also in certain cases of pneumonia, typhoid fever and in diseases of the central nervous system. We have noted a marked alkalinity in certain cases of nephritis, particularly of the severe chronic parenchymatous type with much edema, which renders the examination for casts difficult.

The urine may be alkaline because of the alkaline secretions and exudates of cystitis or urethritis and, lastly, because of alkaline fermentation due to the action of bacteria in the bladder which break up the urea into ammonium carbamate and carbonate. To determine whether the alkalinity of the urine is due to a fixed alkali or to ammonia (in which case, it always is the result of bacterial fermentation) a strip of red litmus paper is wet with the urine and then dried. If the red color returns the alkalinity is due to ammonia. Others moisten red litmus paper with water and hang it in the mouth of the bottle. If much ammonia is present it will turn blue. (Even normal urine contains enough ammonia to turn the paper slightly blue in time.)

The acidity of the urine can be increased, but not beyond a certain point. An increased proteid metabolism will do this or the careful administration of dilute mineral acids. Brown[13] has reported a series in cases of girls and young women of distinctly neurotic temperament with the urine even from 2 to 5 times the normal acidity (phenolphthalein as indicator) and symptoms of cystitis, *i.e.*, pain in the trigonal region, but without demonstrable lesions. He suggests that it is a neurosis of urinary secretion.

The urine in diabetes mellitus is very acid if it contains considerable oxybutyric and diacetic acids. The question of the reaction of the urine in the so-called "uric acid diathesis" has not yet been decided. The reason why it is so difficult to increase the acidity of the urine in the case of man is that his body protects itself against an acid intoxication by increasing the elimination of ammonia, thus protecting from depletion the native mineral alkaline store. The herbivorous animals have this ability to a much less degree and so they are more easily poisoned by acids than is man.

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[13] Phila. Med. Jour., March 2, 1901.

The effect of muscular work on the reaction of the urine is still doubtful.

Some urines after standing for from 6 to 12 hours become more acid because of the so-called " acid fermentation." The reason for this is uncertain. It is inconstant and is always soon succeeded by an alkaline decomposition. Hammarsten considers it due to the reaction between the biurates and MH_2PO_4.

Determination of the Total Acidity of the Urine.—To determine the total acidity of the urine Naegeli [14] added *0.1N* NaOH directly to 10 c.c. of urine, using phenolphthalein as indicator. The error is at least from 4 to 8%. Folin [15] uses potassium oxalate in excess to rule out the error from ammonium salts and calcium phosphate. His method is as follows:

Twenty-five cubic centimeters of urine are measured by a pipet into a 200 c.c. Erlenmeyer flask, 1 or 2 drops of 0.5% phenolphthalein solution added and 15 to 20 gms. of potassium oxalate. The flask is shaken well for 1 minute, then at once titrated with *0.1N* NaOH, shaking all the time. The alkali is added until a faint yet distinct coloration is produced.

The Mineral Acidity of the Urine—Folin's Method.—From 0.3 to 0.6 gm. of pure, dry, granular potassium carbonate is accurately weighed (within an accuracy of 0.2 mgm.) into a platinum dish and 25 c.c. of the urine to be examined measured into it. (If the urine contains much albumin this should be removed by heat and acetic acid. A trace of albumin contains too little sulphur to affect the results appreciable.) The urine is then evaporated on the sandbath or electric oven to dryness and when perfectly dry the contents of the dish are burned at just below red-heat (that is, the dish should never be more than faintly red-hot) over a so-called "radial burner" giving a flame wide enough to heat the entire bottom of the platinum dish. One must be sure the gas used does not contain sulphur. If there is any doubt on this point (which is tested by burning some of the pure potassium carbonate in the platinum dish and testing the contents for sulphates) an alcohol flame may be used. If the entire bottom of the platinum dish is not evenly heated the cyanogen derivates of urea, which resemble mineral matter, will melt, flow to the cooler portions, and escape decomposition.

The burning should continue for about an hour after all ammoniacal fumes have ceased to come off. Then the flame is removed. It makes little difference if the ash is not perfectly white. Just 10 c.c. of hydrogen peroxide water are next added, the dish covered with a watch glass, and gently warmed until the peroxide is decomposed. The watch glass is then removed and the sputterings rinsed into the dish by means of a little water. The contents of the dish are again evaporated to perfect dryness and are again heated over the radial burner as before for about an hour. The hydrogen peroxide is used to oxidize the thiocyanate and any small amount of sulphides which may have formed during the burning. Even with these precautions the complete combustion of the urine is very difficult.

The residue is now dissolved in water with the help of an excess of *0.1N* HCl (75 or 100 c.c., depending on how much carbonate was used), and is rinsed into an Erlenmeyer flask, boiled to drive off the carbonic acid and cooled. The excess of acid is then titrated with *0.1N* NaOH in the presence of a small amount of potassium oxalate (to precipitate the calcium) and 2 drops of a ½% solution of phenolphthalein.

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[14] Zeitsch. f. physiol. Chem., 1900, xxx, 313.

[15] Am. Jour. Physiol., 1903, ix, 265.

The amount of alkali and of acid added to the urine is known, one must determine: (1) the alkaline strength of the potassium carbonate; (2) the acidity of the hydrogen peroxide; (3) the SO_3 content of the hydrogen peroxide; (4) the preformed ammonia in the urine; (5) the inorganic SO_3 of the urine; and, finally, (6) the total SO_3 found in the titrated solution of the urine residue.

The potassium carbonate and hydrogen peroxide will keep for months in well stoppered glass bottles, so the first 3 determinations need be made but once (for any given sample of carbonate and peroxide).

To calculate the result, one subtracts from the apparent excess of acidity found on titrating the burned urine residue the sum of the preformed ammonia, the acidity of the hydrogen peroxide and the acidity due to the organic SO_3 of the urine, all in terms of *0.1N* acid.

The acidity (in c.c. of *0.1N* acid) of the organic SO_3 is obtained by subtracting the sum of the SO_3 of the hydrogen peroxide and the inorganic SO_3 of the urine from the total SO_3 of the urine residue and dividing the amount thus obtained in milligrams by 8 (8 gms. of the organic sulphur, neutral and ethereal, are taken to represent 1 c.c. of *0.1N* acid).

To illustrate: 25 c.c. of urine were burned with 0.5287 gm. of potassium carbonate (7.76 mgms. of which contained 1 c.c. *0.1N* alkali). The burned residue was boiled with 75 c.c. of *0.1N* HCl and the titration required 1 c.c. *0.1N* NaOH. An ammonia determination gave 5.2 c.c. *0.1N* NH_3 in 25 c.c. of urine. The total $SO_3 = 59.9$ mgms.; the inorganic $SO_3 = 42.8$ mgms. (10 c.c. of the hydrogen peroxide used contained 8.8 mgms. SO_3 and 0.5 c.c. *0.1N* acid).

0.5287 gm. K_2CO_3	$=68.1+$	c.c. *0.1N* NaOH
NaOH added	$=19.$	c.c. *0.1N* NaOH
Total alkalinity	$=87.1+$	c.c. *0.1N* NaOH
HCl added	$=75.$	c.c. *0.1N* NaOH
Apparent acidity of urine	$=12.1$	c.c. *0.1N* HCl
Ammonia in 25 c.c. urine	$=5.2$	c.c. *0.1N* HCl
Acidity of H_2O_2	$=0.5$	c.c. *0.1N* HCl
Acidity of organic $SO_3 = \frac{59.9-(42.8+8.8)}{8}$	$=1.$	c.c. *0.1N* HCl
	6.7	

Mineral acidity in 25 c.c. $=12.1-6.7=5.4$ c.c. *0.1N* HCl.

The Organic Acidity of the Urine.—By subtracting the mineral acidity from the total acidity one obtains the "organic acidity," or rather the total equivalence of organic acid whether free or combined. In cases of acid intoxication, as in diabetes, the mineral acidity may turn out to be an alkalinity and all the acidity be organic. In the latter case the mineral alkalinity is added to the total acidity to get the organic acidity.

THE NITROGENOUS BODIES

The Nitrogen Output.—The total nitrogen of the urine is the best index of proteid metabolism. It is indeed fortunate that we have a satisfactory method of determining this, since it is our basal figure in all metabolism work.

Folin[16] studied carefully the nitrogen distribution in the urine of normal men on a nitrogen-rich diet (Table I) and in 1 case on a very low nitrogen diet (Table II).

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[16] Am. Jour. of Insanity, 1905.

	Table I	Table II
Total nitrogen	14.8–18.2 gms.	4.8– 8.0 gms.
Urea-nitrogen	86.3–89.4%	62.0–80.4%
Ammonia-nitrogen	3.3– 5.1%	4.2–11.7%
Creatinin-nitrogen	3.2– 4.5%	5.5–11.1%
Uric acid-nitrogen	0.5– 1.0%	1.2– 2.4%
Undetermined nitrogen	2.7– 5.3%	4.8–14.6%

Hammarsten's [17] figures are the ones quoted in most text-books.

	Normal adults on mixed diet—	Infants
Total nitrogen	10–16 gms.	
Urea	84–91	73–76
NH_3	2– 5	7.8– 9.6
Uric acid	1– 3	3– 8.5
Extractives	7–12	7.3–14.7

The sum of the nitrogens of the urea and the ammonia added together bears a very much more constant relationship to the total N (91 to 93%) than does either one alone.

Total Nitrogen.—While the normal daily output of nitrogen is usually stated to be from 10 to 16 gms. since this is the average output of many healthy persons, yet this amount is considered by some to be evidence of overeating, since men can keep well and even gain weight on a diet which yields a daily output of but 5 or 6 gms. of nitrogen. Taylor's very careful work, continued over long periods of time, on the daily output of nitrogen in normal men, shows how wide are the variations from those limits which have been considered normal. Unfortunately most of the work published on the nitrogen of the urine is of little value since only the urea N was determined or due attention was not paid to the total nitrogen of the food, to the character of the food (its acid- or alkaline-producing qualities), and to the age, nutritional condition and previous diet of the patient. Again, the periods of observation should be at least 7 days long, during which time the diet and the daily amount of water consumed both should be constant. The patient should exercise each day a fairly constant amount. Granted that all these points are carefully watched, even then marked variations in the nitrogen output will be observed.

By " nitrogen balance " is meant the relation of the nitrogen intake to the nitrogen output. The difference between these 2 figures is usually called the " nitrogen lost " and the " nitrogen retained." When the output is just equal to the intake the person is said to be in " nitrogenous equilibrium."

In general, the total urine nitrogen output is increased by increased proteid metabolism, whether by a heavy proteid diet, or by anything increasing the proteid catabolism of the body tissue. It is decreased by a diet rich in carbohydrates, in which case it reaches a lower point than during a fast since in the latter case the body oxidizes more of its tissue proteid.

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[17] Lehrb. d. Phys. Chem., 1899, p. 421.

The total nitrogen output reaches its maximum a few hours after a heavy proteid meal. The evidence that exercise increases it is unsatisfactory since the other differences between day and night urine were not taken into account (see page 90). Hot baths increase the nitrogen output.

Any increase of water elimination will increase that of nitrogen even though the diet is fairly constant. One explanation given for this is that in the renal cells there is always a certain amount of nitrogenous waste which an increased water flow will wash out more thoroughly; others say that the many tissue ferments follow the general law of ferments and act better the more dilute the solution. The output of nitrogen is diminished physiologically by a " poor " diet, by a reduced output of water, after profuse sweating, in pregnancy and after small doses of quinine.

Pathologically the total nitrogen output is increased under the following conditions: in fevers, in which cases it is due not to the temperature *per se*, but, more likely, to the effect upon metabolism of the toxins causing the fever (the exceptions are acute nephritis causing dropsy and diseases with diarrhea or with the formation of large exudates); in diabetes, in mild cases if the patients are on a proteid-rich diet, but more especially in the severe ones who oxidize the protein of their own tissues; after various protoplasm poisons, as arsenic, antimony, phosphorus, etc.; in any condition which diminishes the oxygen intake, as prolonged dyspnea, hemorrhage, carbon monoxide poisoning, etc.; in acute lobar pneumonia during the resolution, that is, while there is autolysis, absorption and excretion of the solid exudate (in a case of Müller's the excess of nitrogen output during the resolution was 28 gms., which represented 800 gms. of pneumonic exudate); during the absorption of exudates or transudates; and, finally by anything which increases the water output, as, for instance, diabetes insipidus, in which disease a daily output of 130 gms. of urea have been reported, and in cases of chronic nephritis with polyuria. The retention of nitrogen may be noted: in persons who are gaining in weight; in myxedema; during the convalescence of fevers (in a case of convalescent typhoid fever reported by Lüthje the patient retained during 26 days 121.38 gms. of N, which would represent 758.6 gms. of albumin or 3568.6 gms. of muscle. This person gained in weight during that time 6490 gms.); and during the last stage of pregnancy. Pregnancy is followed by a diuresis and increased nitrogen output which begins about the second day of the puerperium.[18] The nitrogen output is diminished in all conditions which hinder digestion and the absorption of proteins from the intestine; by those which reduce the oxidization processes in the body, as severe cachexias; by those conditions accompanied by large exudate and transudate formation, as dropsy; and by renal conditions, both organic and functional, which hinder excretion. A marked reduction in the amount of nitrogen excreted is sometimes an early sign of uremia.

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[18] Slemons, Johns Hopkins Hosp. Rep., 1904, vol. xii.

Estimation of Total Nitrogen.—*Gumming's modification of the Kjeldahl method* is quite uniformly used. From 5 to 20 c.c. of the urine, according to its concentration, are measured into a combustion flask of about 250 to 300 c.c. capacity of best quality glass and 15 c.c. of concentrated sulphuric acid, 10 gms. of potassium sulphate, and about 1 gm. of copper sulphate are added. This flask is placed on a proper holder in a hood with a good draft resting on a sheet of asbestos gauze and its contents boiled over a free flame until clear and blue. The worker should be careful when washing down the carbon from the sides of the glass by shaking the fluid not to burn himself. The oxidation may be aided by adding a little $KMnO_4$. After the fluid is perfectly blue the heat should be continued for a few minutes or even for half an hour, that the combustion of uric acid and certain other bodies may be perfect. At the end of this time practically all of the nitrogen will have been converted into ammonia and is therefore present as ammonium sulphate. The fluid is then allowed to cool, distilled water is next added in excess, and the fluid poured into a distilling flask (see Fig. 26, *A*) of 1 liter capacity, with long neck and round bottom. All of the contents of the combustion flask should be washed into this flask by rinsing it 3 or 4 times with distilled water. Talcum powder or zinc granules may now be added to prevent bumping.

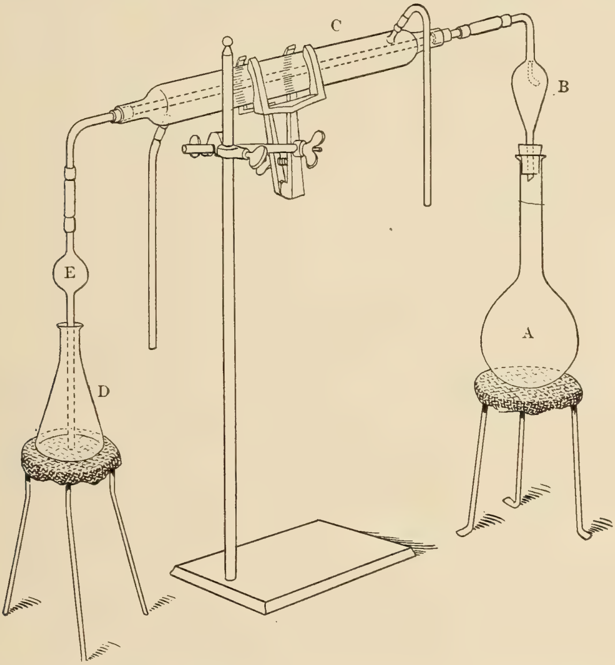

Fig. 26.—Distilling apparatus for nitrogen determination (Kjeldahl). *A*, distillation flask; *B*, safety bulb; *C*, Liebig cooler; *D*, Erlenmeyer flask to receive distillate and containing the standard acid; *E*, safety bulb to prevent back-flow.

That amount of strong sodium hydroxide (specific gravity 1.230) which has been found by previous experiments more than sufficient to neutralize the acid is now added and the flask at once fitted to the Liebig cooler. The lower end of this cooler ends in a bent tube which descends vertically to the bottom of a small Erlenmeyer flask, *D*, of about 300 c.c. capacity, into which have been previously measured 50 c.c. of *0.25N* H_2SO_4. In the subsequent distillation, therefore, all the ammonia, both that given off at once in the cold and that liberated on boiling will bubble through this acid and thus be caught. The distillation is continued until about 100 c.c. of distillate have passed over. The boiling should not be too vigorous, and the apparatus should be protected by a safety-bulb, *B*, and watched that no acid spurt into the cooler. When the distillation is completed the Erlenmeyer flask is lowered and the next few drops of distillate tested with lacmoid paper to make sure that the ammonia has entirely passed over. The acid clinging to the end of the tube is washed into the flask. This sulphuric acid is then titrated against *0.25N* NaOH, using cochineal, methyl orange or pure litmus as indicator. Pure litmus is the best if the necessary precautions are used, but cochineal is sufficiently correct for ordinary work and is the most convenient since it can be used also by artificial light. (The cochineal bugs are ground fine and extracted with 50% alcohol. This filtered extract is used as indicator.) From 50 c.c., the original amount *0.25N* H_2SO_4, are subtracted the number of cubic centimeters of *0.25 N* NaOH necessary to neutralize the acid remaining after the distillation. The difference is the amount of *0.25N* H_2SO_4 neutralized by the ammonia. This value multiplied by 0.0035 gm. is the weight of nitrogen in the amount of urine used.

(NOTE.—This method does not indicate nitrates or nitro compounds.)

In many laboratories the nitrogen of the urine is determined without distillation. The contents of the combustion flask are poured, and the flask rinsed, into the apparatus for the determination of ammonia, the alkali added and the amount of ammonia determined (see page 123).

The estimation of nitrogen in the urine by the *Colorimetric Method of Folin and Farmer* as modified by Myers and Fine and further slightly by Gradwohl and Blaivas[19] requires but a few drops of urine and but from 5 to 10 minutes of time.

The amount of urine used should contain between 0.35 and 0.75 mgms. of nitrogen. One cubic centimeter of urine is measured with an Ostwald-Folin pipet into a 25 c.c. volumetric flask and diluted to the 25 c.c. mark with distilled water. (In the case of urines with a low specific gravity a dilution of 1 to 10 may be sufficient.) After it has been thoroughly mixed 1 c.c. of this diluted urine is measured into a thin glass test-tube and from 5 to 7 drops (0.1 c.c.) of concentrated sulphuric acid, 50 to 100 mgms. of potassium sulphate and a drop of copper sulphate (10%) added. This mixture is now boiled, while it is being shaken continuously, until it becomes dark brown in color and then while the tube is warm, but not hot, a drop of hydrogen peroxide is added and the heating continued for about 1 minute in case the fluid is not clear. The tube is now allowed to cool for 1 minute and its content then washed into a 50 c.c. volumetric flask (*A*) with about 35 c.c. of distilled water. One now measures with an Ostwald-Folin pipet 5 c.c. of an ammonium sulphate solution containing 1 mgm. of nitrogen per 5 c.c. (prepared by dissolving 0.944 gm. of ammonium sulphate in distilled water and making the solution up to 1000 c.c.) into a 50 c.c. volumetric flask (*B*) and adds about 30 c.c. of distilled water.

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[19] Jour. A. M. A., Sept. 9, 1916, vol. lxvii, p. 809.

One now makes a fresh dilution of 10 c.c. of modified Nessler's solution. (This solution is made up by dissolving 100 gms. of mercuric iodide and 50 gms. of potassium iodide, both finely powdered, in a liter volumetric flask containing about 400 c.c. of distilled water. Two hundred grams of KOH are dissolved in 500 c.c. of distilled water, cooled thoroughly, and added, with constant shaking, to the mixture in the flask. This solution is then made up to 1 liter with water. It usually becomes perfectly clear. It is kept at 37° C. in an incubator over night or until the yellowish white precipitate which may settle out is thoroughly dissolved and only a small amount of dark brownish precipitate remains. The solution is now ready to be siphoned off for use.) Next, 10 c.c. of this solution are diluted with 40 c.c. of distilled water, mixed thoroughly and then used to make up to volume the contents of the two flasks *A* and *B*. In the case of flask *B*, the Nessler's solution has neutralized the sulphuric acid. The dry, glass-stoppered wedge of the Hellige colorimeter is now filled with the standard solution and adjusted in the colorimeter. Slightly over 2 c.c. of the unknown solution are now poured into the empty cup, inserted in the colorimeter, and the colors matched, preferably by a North light. The amount of nitrogen in $\frac{1}{25}$ c.c. of urine, the amounts actually used, may be ascertained from Table I (page 110).

Since the figures in the table are given for dilution of 100 c.c. and the dilution here employed is 50 c.c., the result obtained should be divided by 2.

Urea is the nitrogenous body of the urine present in largest amount and the one which until recently has attracted most attention. Since none of the methods formerly employed to determine it[20] gave reliable results, the great mass of research work done using these methods must be considered inaccurate.

The output of urea has been used as a test of the digestion. A meal containing an excess of nitrogen is ingested; for illustration, 500 gms. of meat, 8 eggs and 200 gms. of bread. During this and the following day at least 50 gms. of urea should be excreted.

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[20] Liebig's, Hüfner's, Moerner-Sjoquist, Schoendorf, Folin's, et al.

TABLE I *

ESTIMATION OF NITROGEN WITH THE HELLIGE COLORIMETER

Calorimetric reading	Nitrogen mgms. per dilution of 100 c.c.	Colorimetric reading	Nitrogen mgms. per dilution of 100 c.c.	Colorimetric reading	Nitrogen mgms. per dilution of 100 c.c.
20	1.73	40	1.31	60	0.89
21	1.71	41	1.29	61	0.87
22	1.69	42	1.27	62	0.85
23	1.67	43	1.25	63	0.83
24	1.65	44	1.23	64	0.81
25	1.62	45	1.20	65	0.78
26	1.60	46	1.18	66	0.76
27	1.58	47	1.16	67	0.74
28	1.56	48	1.14	68	0.72
29	1.54	49	1.12	69	0.70
30	1.52	50	1.10	70	0.67
31	1.50	51	1.08	71	0.65
32	1.48	52	1.06	72	0.63
33	1.46	53	1.04	73	0.61
34	1.44	54	1.02	74	0.59
35	1.41	55	0.99	75	0.56
36	1.39	56	0.97	76	0.54
37	1.37	57	0.95	77	0.52
38	1.35	58	0.93	78	0.50
39	1.33	59	0.91	79	0.48

* Myers and Fine's table copied from Gradwohl and Blaivas.

The normal person on an average diet is said to excrete from 20 to 40 gms. of urea each 24 hours; on a poor diet, only 15 or 20 gms.; if on a very rich diet, even 100 gms. in 24 hours. Men are said to eliminate more than women. In general it may be said that a vigorous person will eliminate on an average diet about 30 gms. and an invalid on a liquid diet about 20 gms. per day.

The amount of urea eliminated may be diminished because the output of total nitrogen is diminished or because the nitrogen is excreted in some other form than urea, *e.g.*, as ammonia. One of the most important functions of the liver is to change the ammonia bodies to urea; hence in certain diseases which decrease the liver function the output of urea will diminish and that of ammonia increase until even from 50 to 60% of the total nitrogen is eliminated as ammonia (see page 122). It is also true, however, that some cases with marked gross lesion of the liver will eliminate urea and ammonia in normal percentages. Again, an unusually large per cent. of the nitrogen may be eliminated as ammonia because of acids which are ingested or formed within the body. These must be neutralized and to protect the mineral alkali of the blood and lymph, nitrogen which otherwise would appear as urea will be eliminated as ammonia and so be withdrawn from urea formation. This occurs in diabetes and in cachexias which disturb the absorption or use of carbohydrates.

The interesting question has been raised, Why is there any great excess of urea at all in the urine? There are various answers to this question. One is that urea is the chief nitrogenous ash of nitrogenous food and that a normal American on an " average " American diet should excrete from 20 to 30 gms. of it each day. Another opinion is that the urea represents that part of our nitrogenous intake which is over and above that which we really needed and that man " living rationally " would have very little urea in his urine. Another view, not so radical, is that not all of the split products of protein digestion are resynthesized to protein; that the cleavage liberates the carbonaceous portion of the protein molecule and the various amido acids, some of which are needed then, others not, and that to get a sufficient amount of a great enough variety of these amidobodies a great deal of proteid must be torn down, the most of which must be rejected; that it is this nitrogen which is finally excreted as urea. It is, however, quite definitely established that urea is the only nitrogenous ash which is diminished both absolutely and relatively when the total nitrogen output is diminished and that a man can keep in nitrogenous equilibrium and in good (?) health for a limited time on an astonishingly limited diet (see the table on page 105).

Urea, when pure, crystallizes out in needles or prisms belonging to the tetragonal system, which are colorless, striated, pale, four-sided columns with ends in 1 or 2 oblique planes and which sometimes are hollow. They contain no water of crystallization, are not hydroscopic, and do not change in the air. They are decomposed by heat, liberating ammonia, the decomposition beginning at 100° C. and becoming most active at 130° C.

The *furfurol test,* the most important for urea, is made, according to Schiff, by bringing 1 crystal of urea the size of the head of a pin in contact on a porcelain dish with 1 drop of concentrated aqueous solution of furfurol and adding at once 1 drop of hydrochloric acid (specific gravity, 1.1). A rapid change of colors takes place; first yellow, then green, blue, violet, and in a few minutes a fine purple-violet. Alantoin gives the same test but less intensely and more slowly. An old furfurol solution should not be used since it may give this change of colors even in the absence of urea. Huppert mixes 2 c.c. of concentrated furfurol solution with 4 to 6 drops of concentrated hydrochloric acid. If no red color is produced, 1 crystal of urea is added. In a few minutes the fluid becomes a deep violet color, which gradually turns to black and a black precipitate forms.

The *biuret test* is 1 of the best-known tests for urea. Urea if fused at a temperature of 100° C. gives off biuret and cyanuric acid. To make this test a few crystals of urea in a dry test-tube are heated gently until fluid, then cooled, dissolved in water, made strongly alkaline with NaOH, and then a 2% solution of $CuSO_4$ added drop by drop. A beautiful violet color will be the result.

If one has but a minute quantity of material at his disposal, *e.g.*, a grain of skin frost, the best tests for urea are the *nitric acid* or *oxalic acid* tests. One crystal or 1 drop of the concentrated solution (at least 10%) is allowed to come in contact under the cover-glass with pure nitric acid. At the line of contact the characteristic crystals of urea nitrate $CO(NH_2)_2$ HNO_3 form rapidly as colorless rhombs or hexagonal plates with acute angles which often overlap like shingles. If they crystallize out slowly it is in the form of large, thick, rhombic prisms. These crystals, when heated, volatilize without leaving any residue, an essential point to exclude similarly shaped crystals of the heavy metals. The nitric acid used should be free from nitrous acid which would decompose the urea forming carbon dioxide, nitrogen, and water.

In a similar test oxalic acid is used instead of nitric acid. The urea oxalate, $2CO(NH_2)_2H_2C_2O_4$ formed is less soluble in water than the nitrate, which is an advantage. These crystals are rhombs, hexagons, or plates.

A still better method is to dissolve the urea in the least possible amount of absolute alcohol and to bring this in contact with a concentrated ether solution of oxalic acid or, better still, to use an amyl alcohol solution of both.

QUANTITATIVE DETERMINATIONS OF UREA—*Urease Method of Marshall.* Into each of two 200 c.c. Erlenmeyer flasks are measured 1 or 2 c.c. of toluol. Into 1 is measured exactly 5 c.c. of the urine, the urea of which is to be determined, and 100 c.c. of distilled water. One urease tablet is crushed in a glass mortar, dissolved in about 5 c.c. of water and washed into the second flask using for this purpose in all about 90 c.c. of water. Five cubic centimeters of the urine are then carefully measured into this flask. Both flasks are now tightly closed with corks and their contents agitated. They are then allowed to stand at room temperature for at least 8 hours. If haste is desired 2 tablets may be used instead of 1 and the flasks incubated in the thermostat at 40° C. for 1 hour; or, but 1 c.c. of urine may be used, 2 tablets of urease, 100 c.c. of distilled water and the flasks digested at from 40° to 50° C. for 15 minutes only.

After the proper time of incubation has elapsed the contents of both are titrated with *0.1N* HCl, methyl orange used as indicator, until they assume a distinct pink color. The amount of *0.1N* HCl required to neutralize the specimen containing the urease less the amount required to neutralize the control (the preformed ammonia) will give the urea content of 5 c.c. of urine estimated as ammonium carbonate. One cubic centimeter of *0.1N* HCl will indicate 0.001401 gm. of N and this value multiplied by 2.143 the amount of urea.

Quantitative Determination of Urea by the Urease and Colorimetric Method of Folin.—By this method the urea is converted by the urease into ammonium carbonate, the ammonia then liberated by sodium carbonate in excess and drawn over by aëration into hydrochloric acid. The ammonium chloride formed can be determined colorimetrically by the use of Nessler's reagent.

The urine is diluted 1 to 10 with distilled water, 2 c.c. of this measured into a test-tube of such dimensions that it will easily slip into a 100 c.c. narrow cylinder (Fig. 27, *B*) without lip, about 0.1 gm. of urease added and the contents then incubated for ½ an hour in a beaker of water at 50° C. Two drops of caprylic alcohol or 1 c.c. of amylic alcohol are next added to prevent foaming during aëration.

The apparatus for aëration consists of two 100 c.c. cylinders for each sample of urine. If more than 1 specimen is to be examined, and control determinations always should be made, the 4 cylinders may be run in series. Of the 2 cylinders for each test the 1 is graduated, the other not graduated and both are provided with 2-hole rubber stoppers. Cylinder *A* is graduated and is connected by tube *a* with the suction apparatus. Cylinder *B* is not graduated and is connected with the acid wash bottle (*C*). If more than 1 urine is under examination, cylinder *B* is connected with the short connection tube of the other graduated cylinder *A*. The wash-bottle *C* contains sulphuric acid (10%) and is placed at the end of the outfit to prevent any ammonia in the air from gaining entrance into the system. Tube *a* is bent at right-angles and extends only to a point just within cylinder *A*. The tube *b* which extends almost to the bottom of cylinder *A* ends in a bulb pierced by a number of small holes (made with a platinum wire at white heat while the glass is only moderately hot). Cylinder *B* is connected with *A* by a right-angle tube extending to a point just below the stopper. Its other tube *c* has a straight open end long enough to dip into the test-tube (*E*) while its other end is bent at right angles and serves as connection either with the acid wash bottle or with the other series of cylinders in case more than 1 urine is to be examined. Into cylinder *A* are measured 20 c.c. of distilled water and 2 to 3 drops of 10% hydrochloric acid. This is now closed and cylinder *B* opened. To the digested urine in the test-tube an equal volume of saturated sodium carbonate is added,

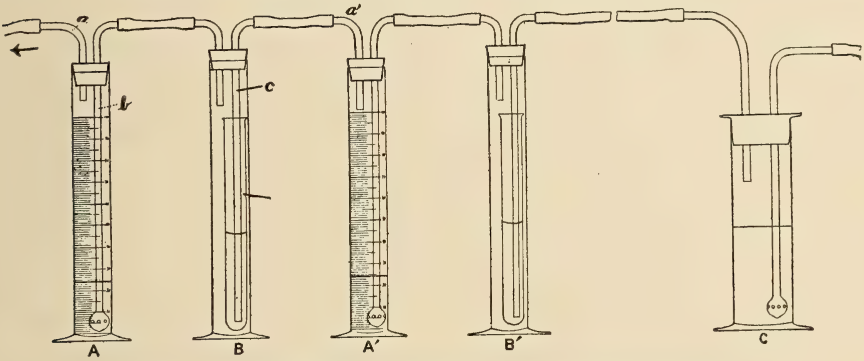


FIG. 27.—Showing the urea apparatus set up and connected to suction.

this being allowed to run slowly down the side of the tube under the urine. the tube is now quickly and carefully placed in cylinder *B* which is then at once closed, care being taken that tube *c* reach almost to the bottom of the test-tube, and all the connections carefully, sealed. The suction, by means of the Chapman pump, is started slowly for about 5 minutes, then increased to the limit and continued for from 30 to 45 minutes. The stopper of cylinder *A* is now removed, care being taken to wash back all fluid on tube *b* with 2 to 3 c.c. of distilled water.

Into a 50 c.c. volumetric flask is pipeted 5 c.c. of an ammonium sulphate solution 5 c.c. of which contain 1 mgm. of nitrogen (see page 109), 25 c.c. of distilled water and 20 c.c. of Nessler's solution (see page 109) diluted 1 to 5. To cylinder *A*, which contains the nitrogen of the urea in the form of ammonium chloride, is added from 10 to 25 c.c. of diluted (1 to 5) Nessler's solution, the amount depending upon the depth of color and this then diluted to 50 c.c., 100 c.c., etc. (depending upon the color). The colorimetric reading should be made at once with the Hellige colorimeter (see page 109) and the result calculated with the aid of Table I.

The result will be the amount of nitrogen in 0.2 c.c. of urine (the urine was diluted 1 to 10 for this test and 2 c.c. of diluted urine taken for the determination).

Suppose the reading was 58. According to Table I, this would indicate 0.93 mgm. per 100 c.c. of a dilution which contained 0.2 c.c. of urine. This multiplied by 5 gives the amount of nitrogen in 1 c.c. of urine; from this should be subtracted the amount of ammonia nitrogen originally present and determined separately. The difference multiplied by the factor 2.14 gives the amount of urea which would contain that amount of nitrogen.

To isolate urea from any solution, all albumin should first be removed. Then the solution, faintly acidified if necessary, is concentrated at a low temperature to a very small volume. Nitric acid is then added in excess, the mixture meanwhile being kept cool. The precipitate is filtered and pressed between filter paper. It is then dissolved in water, decomposed with barium carbonate, dried upon a water-bath and the residue extracted with strong alcohol. The extract is decolorized if necessary with animal charcoal. When this is cooled urea will crystallize out from the warm alcoholic solution.

Uric acid is a substance which has attracted an absurd amount of attention and been the object of a vast amount of time-consuming work. The present consensus of opinion is that it is a specific oxidization product of the nuclein basis and is increased in the urine only as a result of an increase of these bodies in the food, or of an increased metabolism of the tissue nuclei. Horbaczewski believed that it is derived especially from the nuclei of leucocytes. This probably explains but a small fraction of it. It is interesting that in the excrement of birds and certain reptiles uric acid is the most important nitrogenous substance while in that of some carnivora (dogs and cats) little or none can be demonstrated. In the urine of herbivora traces of it are constantly present, while in man it is excreted in a fairly large but still very varying amount. It has been shown that our body has the ability on the one hand to oxidize uric acid and on the other to synthesize it. If hypoxanthin, *e.g.*, be fed a patient, 50% will appear as uric acid. It is probable that in birds, as in mammals, urea is the chief end product of nitrogen metabolism but that their tissues synthesize it further to uric acid, while in mammals it is excreted unchanged. Notwithstanding these opportunities for fluctuation in the amount excreted the recent work tends to prove that uric acid is a very specific product of the oxidization of nuclein bases and that variations in its output would seem to be due in large measure to delays in its elimination.

Uric acid, when pure, is a white crystalline powder consisting of very small prisms or plates. It is with difficulty soluble in boiling water and very little in cold. It is more soluble if not pure. Urea is its best solvent and explains its presence in solution in the urine. It is insoluble in alcohol and ether; is somewhat soluble in hydrochloric acid and in solutions of the alkaline carbonates. The cold solution of uric acid does not redden litmus. It reduces Fehling's solutions when heated, but not Nylander's solution (see page 170). It is decomposed by NaOBr which will liberate about 47.8% of its nitrogen.

The normal output of uric acid in the urine of man varies from 0.2 to 1.25 gms., an average of 0.7 gm. in 24 hours. This represents from 0.5 to 2% of the total nitrogen of the urine. It is increased physiologically by increasing the nucleins of the food. A meal of sweetbreads, *e.g.*, will cause an increase in its output of from 0.5 to 2 gms. in 24 hours. The maximum output occurs from 3 to 5 hours after a meal (that of the nitrogen in 9 hours). There is a relatively large output in the urine of the new-born. In the adult the nitrogen of the uric acid is to the nitrogen of the urea as 1 : 50 to 70, but in the case of the new-born as 1 : 13 to 14.

The amount excreted by a normal person on a mixed diet varies considerably from day to day and the differences between different persons are considerable. Burian and Schur have simplified this problem greatly by showing that the uric acid output may be divided into 2 fractions—the exogenous and the endogenous. By exogenous is meant the uric acid which is formed from the food directly; the endogenous, that part arising from the tissue proteid. The endogenous fraction is therefore the more interesting fraction to consider and in metabolism work involving uric acid the patient should be on a diet, *e.g.*, of eggs and milk, which furnishes sufficient nitrogen and heat but contains no nucleins. Although for this reason most of the quantitative work on uric acid must be discarded yet it is agreed that uric acid is pathologically increased when there is an increased proteid catabolism, as in fevers in which cases the increase of uric acid runs parallel to that of the urea. There is an absolute increase in leukemia, the record output being 8 gms. in 24 hours (reported by Magnus-Levy), although in most cases of this disease it is about 2 gms. per day and its nitrogen is to that of the urea as 1 : 9 (normal 1 : 50-70). While the importance of uric acid in gout is still uncertain, it would seem to be true that between the attacks its output is below normal and that it rises to normal with the acute symptoms. This may help in the diagnosis of a doubtful case of arthritis. The explanation of this would seem to be a retardation in the formation and excretion of uric acid although the large accumulations of biurates in the tophi and around the joints are usually cited as evidence of an increased uric acid production. In other forms of arthritis the question is still unsettled. In diabetes mellitus the increase in uric urine is not marked, only to 2 to 3 gms. per day, and is due to the diet; in pernicious anemia an increase is claimed. In pneumonia during resolution the output is increased, probably because of the breaking down of the nuclei of the cells of the exudate. In cirrhosis of the liver its output is said to be very much increased, Chabria claiming in certain cases even 8 gms. in 24 hours. This is interesting since the liver certainly can synthesize uric acid. The much discussed uric acid diathesis theory so emphasized by Haig and others is still in doubt and is losing ground.

The output of uric acid has been found diminished by a poor diet, in nephritis, during the acute attack of gout, in certain chronic diseases and after large doses of quinine.

It is of interest that when the alloxuric bases are increased in the urine the uric acid decreases in the same proportion.

The *urates* described are:

(1) Neutral, MU, which are not found in nature.

(2) The monoacid- or biurates, MHU, which are gelatinous or crystalline bodies, the best illustration of which are the needles found in gouty tophi.

(3) Quadriurates, MHUU (Roberts), which are easily split to MHU and U by water, heat or acid. They are less soluble than the biurates. They are supposed to make up the common urate sediment. Many observers think the so-called quadriurates are merely mixtures of sodium biurate and uric acid.

The *murexid test* for uric acid is the one in common use. The crystal to be tested is dissolved in 2 drops of nitric acid and evaporated carefully to dryness. The residue will have a beautiful red color. Ammonia is then added, whereupon the color changes to a purple red. Had NaOH or KOH been used in place of the NH_4OH, the color would be more blue and this is an important point in excluding certain other bodies. This test is more brilliant if the nitric acid is evaporated over a water-bath and if the ammonia, not added directly, is placed in a small glass under a bell-jar near that containing the dried residue; also if but little uric acid is used. If the residue is not red but yellow too little nitric acid was used and more should be added and the evaporation repeated. It is an essential part of this test to bleach this color by heat.

Guanin, xanthin, epiguanin, also will give this test, but these are excluded if the substance used was insoluble in an excess of HCl.

In addition to a positive murexid test the ability of the substance in question to reduce Fehling's solution should be tested.

Quantitative Determination of Uric Acid.—*Folin's Method.*—To 300 c.c. of urine are added 75 c.c. of an uranium acetate reagent (consisting of 500 gms. of ammonium sulphate and 5 gms. of uranium acetate dissolved in 650 c.c. of water; 60 c.c. of 10% acetic acid are then added and the volume made up to 1 liter. This solution is to remove the phosphates and certain bodies not well understood whose presence in certain pathological cases disturbs the accuracy of the method). The urine thus treated is well stirred, allowed to stand for 5 minutes and filtered through a double folded filter. Of this filtrate 125 c.c. (representing therefore 100 c.c. of urine) are measured into each of 2 beakers, 5 c.c. of concentrated ammonia are added to each and the beakers set aside until the next day to allow the precipitate of ammonium urate to settle. The clear fluid is then decanted through a filter paper and the precipitate finally collected on this paper and washed with a 10% solution of ammonium sulphate until the filtrate is almost chlorine-free. (This is tested by adding to a little of the filtrate HNO_3 till it is strongly acid and then a drop of $AgNO_3$.) The filter paper is then pierced and the ammonium urate washed into a beaker, using for this purpose about 100 c.c. of water. Fifteen cubic centimeters of concentrated sulphuric acid are then added and the solution titrated while still hot with *0.05N* $KMnO_4$ solution until the first blush of red persists for a few seconds throughout the whole volume of fluid. Each cubic centimeter of the reagent used indicates 3.75 mg. of uric acid. It is necessary to add as a correction 3 mg. of uric acid per 100 c.c. of urine.

A *0.05N* $KMnO_4$ solution is one of such concentration that 1 liter would contain 0.05 gm. of available oxygen with which to oxidize the uric acid. Such a solution would contain therefore 1.581 gms. of recrystallized $KMnO_4$ in 1 liter of water. Since $KMnO_4$ cannot be weighed with sufficient accuracy it is best to make a slightly more concentrated solution, to boil this, which renders it more permanent and then titrate it against a *0.1N* solution of oxalic acid (6.3 gms. per liter) or 1 of potassium tetraoxalate (8.41 gms. per liter). Ten cubic centimeters of this oxalic acid solution are diluted to 100 c.c. with distilled water, 15 c.c. of concentrated sulphuric acid are then added, which will produce a temperature of about 60° C. and the potassium permanganate solution added drop by drop from a buret until a uniform red color remains about 30 seconds throughout the entire volume of fluid. The permanganate solution is then so diluted that 10 c.c. of the oxalic acid will require 20 c.c. of the $KMnO_4$ solution to produce this end reaction.

It is interesting that at the beginning of the titration of uric acid the red color remains longer than later. This is due to the fact that the combustion of the uric acid is much promoted by the increasing percentage of the sulphate of manganese. The color is not permanent owing to the presence of other reducing bodies in the urine and the student, to use the test satisfactorily, should standardize his own solutions, that he may know what to consider the end reaction.

To obtain oxalic acid sufficiently pure it is necessary to recrystallize it 2 or 3 times from a cold saturated solution; or, better, to recrystallize it first from hot dilute HCl (10 to 15%), then from hot alcohol and then from water. The aqueous solution must be heated till the odor of ethyl oxalate has passed off. Oxalic acid cannot be dried in a desiccator or on a hot-air bath.

If from the urine to be examined some uric acid or urates have already precipitated, this sediment should be redissolved by warming the urine, or by the addition of a little saturated lithium carbonate solution and the urine well shaken before it is used.

Colorimetrical Method of Determination.—Into a 15 c.c. conical centrifuge tube one pipets 2 c.c. of urine, adds 15 drops of ammoniacal-silver magnesium mixture, inverts the centrifuge tube in order to mix its contents and then allows it to stand for about 10 minutes in a refrigerator. At the end of this time the tube is centrifugalized for from 3 to 5 minutes and the supernatant fluid poured off by inverting the tube and wiping its lip with filter paper. The ammonia is now removed from the precipitate by volatilization by attaching the mouth of the tube to the suction apparatus.

From this point on the student must work as fast as possible as the colors may fade or the solution become turbid.

One now makes up a standard solution in a 50 c.c. volumetric flask by pipeting into it 5 c.c. of standard uric acid solution (5 c.c. of which equals 1 mgm. of uric acid) adds 2 drops of a 5% solution of potassium cyanide, 2 c.c. of Folin-Macallum reagent, 20 c.c. of saturated sodium carbonate and in 1 minute fills up with water to the 50 c.c. mark.

To the precipitate in the centrifuge tube (which is now free from ammonia) are added 2 drops of a 5% solution of potassium cyanide, the tube shaken so as to dissolve the precipitate, then 2 c.c. of Folin-Macallum reagent and the contents of the tube washed into the 100 c.c. graduate with from 15 to 20 c.c. of saturated sodium carbonate solution (with 20 c.c. if the color is well developed, 15 c.c., if fainter). Since it is quite important that this, the unknown solution, be weaker in color than the standard, one now waits for from 40 to 60 seconds before determining from the depth of color whether to dilute it to 50 c.c. or 100 c.c.

The readings are then made with the Hellige colorimeter using Table II to estimate the amount of uric acid present.

TABLE II *

ESTIMATION OF URIC ACID WITH HELLIGE COLORIMETER

Calorimetric reading	Uric acid mgms. per dilution of 100 c.c.	Calorimetric reading	Uric acid mgms. per dilution of 100 c.c.	Calorimetric reading	Uric acid mgms. per dilution of 100 c.c.
20	1.67	40	1.28	60	0.88
21	1.65	41	1.26	61	0.86
22	1.63	42	1.24	62	0.84
23	1.61	43	1.22	63	0.82
24	1.59	44	1.20	64	0.81
25	1.57	45	1.18	65	0.79
26	1.55	46	1.16	66	0.77
27	1.53	47	1.14	67	0.75
28	1.51	48	1.12	68	0.73
29	1.49	49	1.10	69	0.71
30	1.48	50	1.08	70	0.69
31	1.46	51	1.06	71	0.67
32	1.44	52	1.04	72	0.65
33	1.42	53	1.02	73	0.63
34	1.40	54	1.00	74	0.61
35	1.38	55	0.98	75	0.59
36	1.36	56	0.96	76	0.57
37	1.34	57	0.94	77	0.55
38	1.32	58	0.92	78	0.53
39	1.30	59	0.90	79	0.51

* Myers and Fine's table copied from Gradwohl and Blaivas.

Example 1.—Suppose the dilution is to 100 c.c. and the reading 60. The equivalent of 60 as given in the table is 0.88 mgm. in the amount of urine which was diluted to 100 c.c., *i.e.*, in 2 c.c. of urine. One cubic centimeter of the urine contained therefore 0.44 mgms. of uric acid, and since uric acid contains 33% nitrogen, 0.1452 mgms. of N.

The ammoniacal-silver magnesium mixture is made up by mixing 70 c.c. of 3% silver nitrate solution, 30 c.c. of magnesium mixture and 100 c.c. of concentrated ammonia. Any turbidity which may develop is removed by filtration.

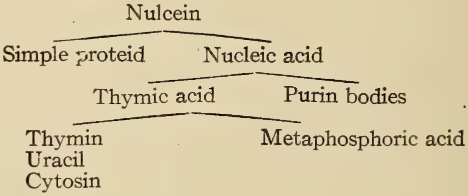
This magnesia mixture is made by dissolving 35 gms. of magnesium sulphate and 70 gms. of ammonium chloride in 280 c.c. of distilled water and then adding 140 c.c. of concentrated ammonia.

For the preparation of uric acid standard solution, one dissolves 9 gms. of pure crystalline hydrogen disodium phosphate and 1 gm. of dihydrogen sodium phosphate in from 200 c.c. to 300 c.c. of distilled water, filters and makes up to about 500 c.c. with hot distilled water. This warm, clear solution is now poured on 200 mgms. of pure uric acid (Kahlbaum) suspended in a few cubic centimeters of water in a liter flask. The flask is agitated until the uric acid is completely dissolved and exactly 1.4 c.c. glacial acetic acid at once added, the solution made up to 1 liter, mixed, and 5 c.c. of chloroform added. Five cubic centimeters of this solution are equivalent to 1 mgm. of uric acid. This solution should be freshly prepared every 2 months. Before weighing out the 200 mgms. of uric acid, it is well to dry the bulk of the uric acid from which the above amount is to be weighed in a drying oven overnight at 100°.

For the preparation of the Folin-Macallum reagent, one boils 100 gms. of sodium tungstate, 20 c.c. of concentrated hydrochloric acid and 30 c.c. of 85% phosphoric acid in 750 c.c. of distilled water for 2 hours and then makes it up to 1000 c.c. During the boiling it is well to have a funnel over the flask so as to prevent undue evaporation.

Rudisch and Kleeberg[21] have reported a method for determining uric acid and the purin bases which they think superior in accuracy even to the Ludwig-Salkowski method and so simple that it can be used clinically. They precipitate all these by an excess of *0.02N* $AgNO_3$, and then determine the excess of silver volumetrically by titration with *0.02N* KI. The end reaction is recognized by testing the mixture in test-tubes after the addition of each successive portion of KI with nitrous-sulphuric acid (25 c.c. H_2SO_4 to 75 c.c. H_2O, then 1 c.c. of fuming HNO_3) and starch solution, until the blue of starch-iodine compound appears. The separation of uric acid from the other purin bodies depends on the solubility of the silver compounds of the latter in strong ammonia solutions.

The Purin Bases.—The purin, alloxuric, xanthin, or nuclein bodies, as they have been called, are found in the urine in very small amounts. These bodies all are compounds of the purin nucleus combined with the amido, oxy, and methyl groups. Of those present in the urine some are exogenous that is, are derived wholly from food, but others are of endogenous origin, *i.e.*, are end products of the clearage of the tissue nucleins. Their formation is represented by the following diagram:



The most important of the purin bodies are xanthin and hypoxanthin. Among the others are adenin, episarkin, and epiguanin. Some have claimed to have found guanin and carnin in the normal urine but that is as yet unconfirmed. That amount of each which appears in the urine is the residue which has escaped transformation to uric acid. The 3 which make up the bulk of the purin bodies found in the urine are heteroxanthin, paraxanthin and methylxanthin, and these are exogenous in origin, that is, are derived directly and wholly from the caffeine, theobromine, and theophylin of the food. The total amount of purin bodies found in the urine varies from 15.6 to 45.7 mgms. in 24 hours. Others consider that, for a mixed diet, 87 mgms. is an average output (Camerer), 44 mgms. that for a meat and 111 mgms. for a vegetable diet.

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[21] Am. Jour. of Med. Sci., 1904, vol. cxxviii, p. 899.

XANTHIN occurs normally in the urine in minute traces. From 10,000 liters of normal urine 16 gms. of xanthin have been isolated. Very rarely it is the chief constituent of a urinary sediment or even of a calculus, several of which have been described. It is increased in leukemia, in the nephritis of children (in which cases even 28.5 mgms. per 100 c.c. instead of, as normally, 3.8 mgms. have been reported).

The principal test of xanthin is *Weidel's Test.* The substance in question is boiled in a test-tube with hydrochloric acid and a little $KClO_3$. It is then carefully evaporated to dryness, and the residue moistened with ammonia. A red or a purple-violet color results. Another test is to add HNO_3 and evaporate to dryness in a porcelain dish, which will produce a yellow residue. On the addition of NaOH and warming, this becomes a purple-red color.

GUANIN is said to have been found in the urine, especially in leukemia. It gives the same test with nitric acid as xanthin, excepting that the addition of the alkali produces a more blue-violet color. It does not give the Weidel reaction.

HYPOXANTHIN is present in the normal urine and in considerable amounts in leukemia. It gives neither the nitric acid nor the Weidel tests.

ADENIN occurs in urine, especially in leukemia. The characteristic reaction of this substance is that if its crystals be warmed slowly in an amount of water insufficient to dissolve them, when the temperature reaches 50° C. there will appear a sudden cloud. It does not give the nitric acid nor the Weidel test. Its other reactions are the same as hypoxanthin.

The best method of the quantitative determination of these bodies is that of Salowski. From 400 to 600 c.c. of urine (albumin removed) are precipitated by a magnesium mixture and filtered. The filtrate is then precipitated with a 3% ammoniacal silver solution (6 c.c. per 100 c.c. of urine) and filtered. This silver precipitate is washed thoroughly and then suspended in about 600 to 800 c.c. of water, slightly acidified with hydrochloric acid and decomposed with H_2S. The fluid is then heated to boiling and filtered hot. The filtrate is evaporated on a bath to dryness and the residue extracted with 3% hot sulphuric acid, from 25 to 30 c.c. being used. The extract is allowed to stand for 24 hours.

The uric acid is then filtered out and washed, the filtrate made alkaline and again precipitated with $AgNO_3$. This precipitate is then collected on a small chlorine-free filter, washed, dried and carefully ashed, the ash dissolved in nitric acid and titrated for chlorides by the ordinary Volhardt method. One part of silver equals 0.277 parts of the xanthin base nitrogen, or 0.7381 parts of the xanthin bases. The uric acid can be determined in the same portion.

The enormous literature on the xanthin bases has lost its value since some of the methods used have been found incorrect. It is quite certain, however, that in leukemia these bodies are increased, also in tuberculosis; and that their output bears a reciprocal relation to that of uric acid.

Ammonia.—The figures usually given for the 24-hour output of ammonia in normal urine (from 0.3 to 1.2 gms., average 0.7 gm.) are considered by Taylor to be much too high. He found that if the urine be carefully protected from all decomposition only about 1/10 of the above amount will be present. Under normal conditions the elimination of ammonia reaches its maximum during sleep—that is, when digestion is at rest. Many believe that the ammonia in normal urine is that which has been withheld from urea formation in order to balance acid ions. But this theory cannot explain all, for some will be present even after long continued alkaline medication.

Ammonia is one of the most important end-products of proteid metabolism. In the arterial blood there is 0.4 mgm., and in the portal blood 1.85 mgm. in 100 c.c. (Hordynski). It is found in all the tissues, especially in the stomach wall which contains 36.4 mgms., and the intestinal wall which contains 32.4 mgms. per 100 gms. of these tissues. It is especially abundant in these organs at the height of digestion. In the other organs the amount is more constant. Under normal conditions the ammonia bodies, all of which are rather toxic, are rapidly synthesized to urea, by the liver especially, therefore in certain hepatic diseases—*e.g.*, in far-advanced cirrhosis and cancer—while the total nitrogen output remains unchanged the percentage of urea tends to fall and that of ammonia to rise.

The relation of N : NH_3 is quite constant if the diet is constant and is not affected by the amount of proteid consumed. If much fat be added, however, the percentage of NH_3 is increased. During the secretion of the HCl of the gastric juice the percentage of nitrogen in the urine rises.

The ammonia of the urine is increased: by the ingestion of inorganic acids and of organic acids which cannot be further oxidized, as well as by any increase in the amount of acids which arise in the body and they do this by using ammonia as a base before it can be changed to urea. Man and the carnivora, it may be said with truth, are constantly defending themselves against an excess of acid radicals produced by the catabolism of animal proteids and they do this by using ammonia as a base, thus protecting their native alkalinity from depletion. The herbivora protect themselves less well than man and so suffer more quickly. It is increased by a diet rich in protein, or fat; in conditions producing oxygen starvation; in fever, during the febrile stage and continuing into the convalescence (Rumpf); in diabetes mellitus, in which cases oxybutyric and diacetic may be demonstrated in the urine and the amount of ammonia may vary from 8 to 12 gms. in 24 hours and represent from 25 to 40.4% of the total urine nitrogen; in periodic insanity, in which Edsall found it markedly reduced before the attack and increased just as the attack came on; in certain severe cases of liver cirrhosis since the liver is no longer able to change it to urea; in some cases of the pernicious vomiting of pregnancy in which the ammonia may represent even from 20 to 45% of the total urine nitrogen, while in cases of nervous or reflex vomiting, and in eclampsia there may be no marked increase. (Definite hepatic lesions are found at autopsy in such cases.) And, finally, in normal pregnancy the ammonia percentage is somewhat increased and reaches its maximum during labor.

Quantitative Determination of Ammonia.—*The Schlösing method* as modified by Schäffer is simple and fairly accurate, but too time consuming. (see Fig. 28). The apparatus consists of a wide crystallizing dish or wide Petri's dish, *B*, from 15 to 17 cm. in diameter so that the urine need not be over 2 mm. deep. This rests on a thick glass plate with accurately ground surface. Above the dish of urine, on a triangle, is another dish, *C*, into which have been previously measured 20 c.c. of 0.1*N* H_2SO_4. These dishes are to be covered by a bell-jar the cavity of which is to be made air-tight by greasing its edge and the glass plate thoroughly.

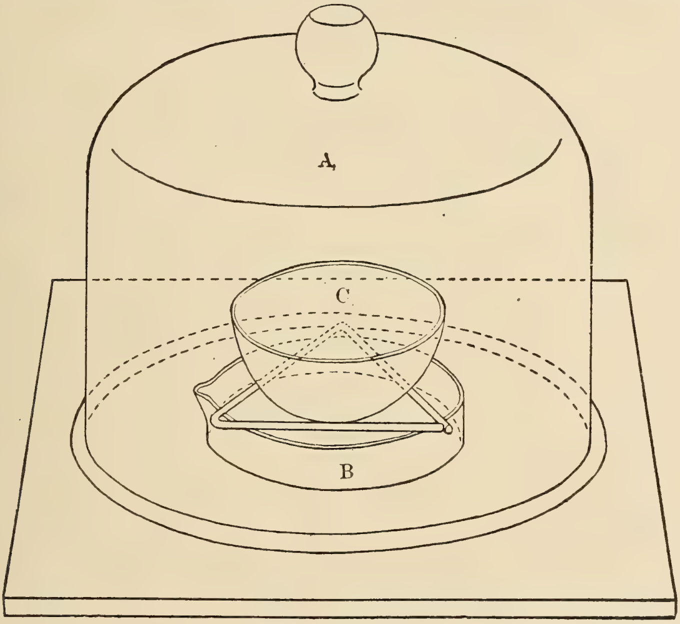

Fig. 28.—Ammonia determination, Schlösing's method. *A*, bell-jar; *B*, dish containing urine; *C*, dish containing acid.

To 25 c.c. of filtered urine measured into the dish *B*, are added 0.5 gm. of sodium carbonate plus an excess of sodium chloride. The sodium carbonate will not split off ammonia from any of the other nitrogenous compounds, as for instance urea, and the sodium chloride will prevent decomposition.

As soon as the alkali has been added the dishes are covered by the bell-jar and the apparatus then not disturbed for 3 or 4 days, or 48 hours if the apparatus be kept at 38° C., during which time the sodium carbonate will have set free all of the ammonia and the sulphuric acid will have taken it up. At the end of this time the sulphuric acid is titrated against 0.1*N* sodium hydroxide to determine the amount of acid which has been neutralized by the ammonia. This figure multiplied by 1.7 mgms. equals the weight of the ammonia originally in the 25 c.c. of urine. If any moisture is visible on the inside of the bell-jar the reaction of this should be tested with litmus and, if alkaline, the entire inner surface of the bell-jar should be washed with distilled water into the sulphuric acid before this is titrated.

Folin's Method.—Folin's method is by far the best yet proposed for the determination of ammonia.

Twenty-five cubic centimeters of urine are measured into an aërometer cylinder (see Fig. 29) from 30 to 45 cm. high and about a dram of dry sodium carbonate added. The further addition of from 5 to 10 c.c. of crude petroleum will prevent foaming.

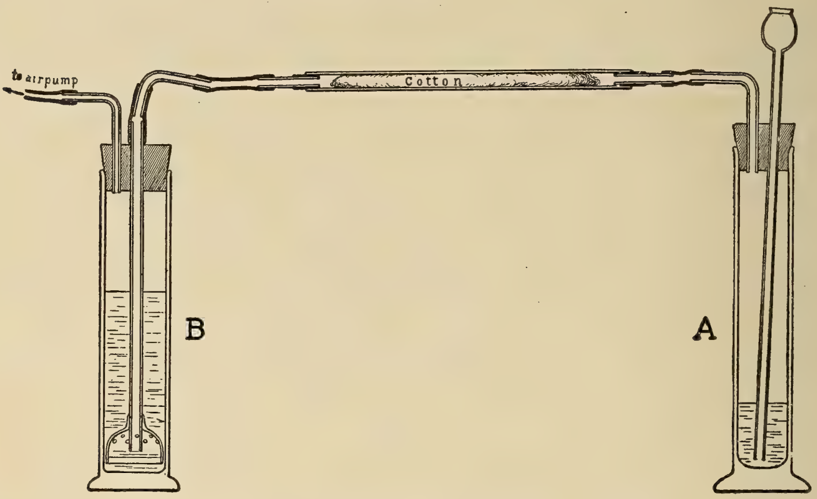

FIG. 29.—Folin's apparatus for ammonia and acetone determination. *A*, narrow tube for urine, connected by a tube containing cotton, with *B*, the cylinder containing acid.

The upper end of this cylinder is then closed by a doubly perforated rubber stopper through which pass 2 glass tubes, only 1 of which is long enough to reach below the surface of the liquid. The shorter tube (about 10 cm. in length) is connected with a glass tube which extends to the bottom of cylinder *B* (capacity about 500 c.c.) which contains 20 c.c. of $0.1N$ H_2SO_4, 200 c.c. of water, and 2 drops of a 1% solution of alizarin red as indicator. The special absorption device designed by Folin and pictured in Fig. 29 compels a very intimate contact of the air containing the ammonia with the acid through which it passes. The absorption bottle is now attached to a good filtering pump which can suck a strong current of air. The air passing through the alkaline urine and then through the standard acid will in about one and a half hours transfer every trace of ammonia to the acid. Its amount is then determined by direct titration with $0.1N$ NaOH, titrating to a red and not to a violet color. In titration involving ammonia phenolphthalein should not be used as indicator, but rather alizarin red, cochineal or a dilute solution of hematoxylin.

Colorimetric Method.—The amount of urine to be used in this determination should contain from 0.75 to 1.50 mgms. of ammonia nitrogen. While in the case of average normal urines 2 c.c. will be about the right amount, this will vary from 5 c.c. of very dilute to less than 1 c.c. of concentrated urines. The desired amount is measured with a pipet into a test-tube about 200 mm. in length and of such diameter that it will slip easily into a 100 c.c. narrow cylinder (*B*, Fig. 27).

The apparatus used is that described on page 113 (see Fig. 27). Here also cylinder *A* contains 20 c.c. of distilled water and 2 to 3 drops of 10% hydrochloric acid. To the urine in the test-tube is added 1 c.c. of amylic alcohol or 2 to 3 drops of caprylic alcohol (to prevent foaming) and then from 3 to 5 c.c. of saturated sodium carbonate solution which is to run gently down the tube under the urine so that none of the ammonia will escape. The test-tube is then quickly placed in cylinder *B* and the stopper quickly inserted with tube *c* reaching almost to the bottom of the test-tube *E*. After the apparatus is properly connected the suction through the apparatus is started slowly and the speed gradually increased so that at the end of about 5 minutes the air current is as rapid as the apparatus will stand. Aëration will be complete in from 15 to 20 minutes. The apparatus is then disconnected and cylinder *A* used for the final determination, after any acid clinging to tube *b* has been washed back into the cylinder with 2 or 3 c.c. of distilled water.

The standard solution is made up in a 50 c.c. volumetric flask as described on page 114.

To the acid in cylinder *A*, which contains the ammonia of the urine, is added from 15 to 25 c.c. (depending upon the depth of color) of diluted Nessler's solution (1 to 5), and this diluted to 50 c.c., 100 c.c., etc., according to the depth of color. The colorimetric reading should be made at once. The calculation is made using Table I (page 110) and the results recorded as in ammonia nitrogen.

Example.—Suppose that 2 c.c. of urine were used, that the final dilution was to 100 c.c. and that the reading was 69. This would indicate 0.70 mgm. of N in 2 c.c. of urine, or 0.35 mgm. of ammonia N in 1 c.c. of urine

Creatinin.—Creatinin, the aldehyde of creatin, is found in normal urine; creatin seldom. In general its origin is the muscle ingested as food and the catabolism of body muscle. Its excretion runs roughly parallel to that of urea; it is increased if the meat of the diet is increased and diminishes during fasting periods. Some claim that it is increased by excessive muscular work only, others (Edsall) that it is increased by all muscular exercise, and is diminished in diseases associated with extensive muscular paralysis, as well as by all conditions which markedly decrease the use of the muscles; that while it is not a perfect index of the condition of muscle metabolism, yet it is the best we have. Normally the output of creatinin in the urine is about 1 gm. a day. Folin[22] has shown that while the creatinin output of different normal persons on a meat-free diet varies widely yet that of each individual is an almost constant quantity which is quite independent of quantitative changes in the total amount of urine nitrogen. He found that moderately corpulent persons eliminated each 24 hours about 20 mgms. of creatinin and lean persons about 25 mgms. per kilo of body weight. Folin also believes[23] that biologically at least creatin and creatinin are not related; that creatinin is a waste product, while creatin is treated as a food. Amberg and Morrill[24] found in the urine of the new-born a small but constant amount of creatinin. McCrudden[25] found in 3 cases of intestinal infantalism that the creatinin was more irregular than normal, that creatinin coefficient was low, and that creatin was present in the urine even when absent from the food.

Jaffé's Test.—To the urine at room temperature is added a little aqueous solution of picric acid and a few drops of dilute NaOH. An intense red color at once develops which increases in intensity and then remains constant for hours. If acid be added the color becomes yellow. Acetone should previously have been removed by boiling. Glucose under these conditions gives, if warmed, a red color. The test is positive for creatinin in solutions of 1 to 5000 or more.

Weyl's Test.—To the urine are added a few drops of very weak sodium nitroprusside (sp. gr. 1.003) and then a few drops of weak NaOH. A ruby-red color appears which soon changes to yellow. If acetic acid be next added and the fluid heated this color will change to green and then to a Berlin blue. This is positive for 0.6 gm. of creatinin per 1000 c.c. of urine. Acetone, if present, should be removed by boiling, for it would give a similar color, which, however, on the addition of acetic acid would change to a cherry-red or purple-red color.

The most important compound of creatinin is its zinc salt $(C_4H_7N_3O_2)_2$-$ZnCl_2$. Creatinin on long boiling will decolorize Fehling's solution and after still longer boiling, if an excess of copper is present, will precipitate yellow $Cu_2(OH)_2$. It is to avoid this error when testing urine with Fehling's solution for glucose that we limit the time of boiling. Creatinin will not reduce a bismuth solution and this is one of the advantages of Nylander's test for glucose.

Quantitative Determination of Creatinin—*Folin's Quantitative Method.*[26]—This determination is based on the color reaction which creatinin (and no other normal urinary constituent) gives with picric acid in alkaline solution. Any fairly good colorimeter will suffice.

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[22] Am. Jour. of Phys., 1905, vol. 13.
[23] Festschrift f. Olaf Hammarsten, iii, 1906.
[24] The Jour. of Biol. Chem., 1903, vol. iii, No. 4.
[25] Jour. of Exper. Med., Feb. 1, 1912, vol. xv, p. 107.
[26] Am. Jour. of Phys., 1905, vol. 13, p. 48.

The reagents necessary are: a *0.5 N* solution of potassium bichromate (which will contain 24.55 gms. per liter); a saturated picric acid solution (containing about 12 gms. per liter) and a 10% solution of sodium hydrate.

Ten cubic centimeters of urine are measured into a 500 c.c. volumetric flask, 15 c.c. of the picric acid and 5 c.c. of the sodium hydrate solutions are then added, and the mixture is allowed to stand for 5 or 6 minutes.

This interval is used to pour a little of the bichromate solution into each of the 2 cylinders of the colorimeter. The depth of the solution in 1 of the cylinders is then accurately adjusted to the 8 mm. mark. With the solution in the other cylinder a few preliminary colorimetric readings are made simply for the sake of insuring greater accuracy in the subsequent readings of the unknown solution. No 2 readings should differ more than 0.1 or 0.2 mm. from the correct value (8 mm. since both cylinders contain the same fluid) if we leave out of consideration the very first reading made, which is sometimes less accurate. Four or more readings should be made in each case and an average taken of all but the first. After a while one becomes sure of the true point, and can take the average of the first 2 readings.

At the end of 5 minutes the contents in the 500 c.c. flask are diluted up to the 500 c.c. mark, the bichromate solution is thoroughly rinsed out of 1 of the cylinders by means of the unknown solution in the flask and several colorimetric readings then made at once.

The calculation of the result is very simple. It has been determined experimentally that 10 mgms. of perfectly pure creatinin will give under the conditions of the determination 500 c.c. of a solution of which a column 8.1 mm. deep will have exactly the same colorimetric value as one 8 mm. deep of a *0.2N* bichromate solution. If then for example it takes 9.5 mm. of the unknown urine-picrate solution to equal the 8 mm. of the bichromate, then the 10 c.c. of urine contain $10 \times \frac{8.1}{9.5} = 8.4$ mgms. of creatinin. If the 10 c.c. of urine examined are found to contain more than 15 mgms. or less than 5 mgms. of creatinin the determination should be repeated using correspondingly different amounts of urine in making up the 500 c.c. of fluid to be tested, since outside of these limits the results are much less accurate. This determination takes less than 15 minutes.

Colorimetric Quantitative Determination of Creatinin.—Into a 100 c.c. volumetric flask or cylinder one measures with a pipet 2 c.c. of the urine to be examined. To this are added 3 c.c. of saturated picric acid and 1 c.c. of 10% sodium hydroxide, they are mixed thoroughly and allowed to stand for 5 minutes in order to allow the color to develop. At the end of this time the mixture is made up to 100 c.c. with tap water, thoroughly mixed and several readings made in the colorimeter, using as standard normal bichromate solution (made by dissolving 49.12 gms. of potassium bichromate in distilled water and making the solution up to 1 liter). The amount of creatinin in 2 c.c. of urine is estimated by the use of Table III. If, *e.g.*, the reading is 58, it indicates that the 2 c.c. of urine used and diluted to 100 c.c. contain 1.62 mgms. and 1 c.c., 0.81 mgm. of creatinin. The creatinin-nitrogen would be 37.2% of this. If the concentration of creatinin in the urine is such that the readings do not fall within the figures of the table, the test is repeated using larger or smaller amounts of urine as the case may be.

TABLE III *

ESTIMATION OF CREATININ WITH THE HELLIGE COLORIMETER

Colorimetric reading	Creatinin mgms. per dilution of 100 c.c.	Colorimetric reading	Creatinin mgms. per dilution of 100 c.c.	Colorimetric reading	Creatinin mgms. per dilution of 100 c.c.
20	2.46	35	2.13	51	1.78
21	2.43	36	2.10	52	1.76
22	2.41	37	2.08	53	1.74
23	2.39	38	2.06	54	1.72
24	2.37	39	2.04	55	1.69
25	2.35	40	2.02	56	1.67
26	2.33	41	1.99	57	1.65
27	2.30	42	1.97	58	1.62
28	2.28	43	1.95	59	1.60
29	2.29	44	1.92	60	1.57
30	2.24	45	1.90	61	1.54
31	2.21	46	1.88	62	1.51
32	2.19	48	1.85	63	1.48
33	2.17	49	1.83	64	1.45
34	2.15	50	1.81	65	1.42

* Myers and Fine's table copied from Gradwohl and Blaivas.

Creatin.—For the *quantitative determination of creatin*, one measures 2 c.c. of urine into a medium-sized test-tube and adds 2 c.c. of normal hydrochloric acid and a very little powdered metallic lead. The contents of the tube is now boiled nearly to dryness over a free flame and then washed with as little water as possible through a small cotton or glass-wool filter into a 100 c.c. volumetric flask. This removes the metallic lead which also reacts with the picric acid and alkali. All the creatin is now in the form of creatinin. To the fluid in the volumetric flask one now adds 3 c.c. of saturated picric acid and 2 c.c. of 10% sodium hydroxide, mixes thoroughly and allows the solution to stand for 5 minutes. Then the mixture is made up to 100 c.c. with tap water, mixed thoroughly and read several times in the colorimeter, using the same standard solution (normal bichromate) and table as for creatinin. The result obtained is the total creatinin. The difference between the performed and the total creatinin gives the creatin in terms of creatinin, or, by multiplying this value by 1.16, the weight of the creatin.

Oxyproteinic and Alloxyproteinic Acids.[27]—Oxyproteinic acid was isolated by Gottlieb and Bondzynski, and alloxyproteinic acid by Bondzynski and Panek. It is claimed that these acids contain all or nearly all of the neutral sulphur of the urine (they contain about 6% of sulphur) also that the oxyproteinic acid explains Ehrlich's Diazo reaction (see page 157). These acids are said to stand the nearest to proteid of all the products of proteid metabolism and yet they give none of the proteid reactions. These writers claim that the normal urine contains about 1.2 gms. of the alloxyproteinic acid per day and about 3 times that amount of oxyproteinic acid. These acids have not been sufficiently studied as yet, and already some have been unable to confirm this work.

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[27] Bondzynski and Panek, Ber. d. chem. Gesell., 1902, vol. xxxv, p. 2959.

THE INORGANIC ACIDS AND BASES

The chlorides make up the bulk of the inorganic matter of the urine. In terms of the sodium salt the urine contains each 24 hours from 10 to 15 gms. of sodium chloride. Practically all of the chlorine of the urine is present in inorganic combination, little if any in organic compound.[28]

The source of the chlorides of the urine is the food. Starvation will reduce them to a trace. More is excreted during the day than during the night. They are increased by anything which increases the amount of urine and also by active exercise. They are diminished during a period in which there is a loss of fluid to the body from diarrhea or vomiting and during the formation of a transudate or exudate and are increased while these are reabsorbing. In acute fevers the chlorides are usually diminished during the fastigium and increase during and after the defervescence. In acute lobar pneumonia there may not be a trace in the urine for several days before the crisis. The practically complete retention of chlorides has little or no prognostic value but has considerable for diagnosis since in a case of doubtful fever it always suggests acute lobar pneumonia. After the crisis the output soon returns to normal. The explanation of this phenomenon in pneumonia is not clear. It is not due to the diet, nor to lack of absorption from the bowel (for chlorides injected subcutaneously are retained) nor is all retained in the exudate. The chlorides in the blood are not increased but are in the other fluids of the body and are fixed in the tissues, even to 4 times the normal amount.

With the febrile crisis of pneumonia the chlorides in the urine suddenly increase, even producing a "chlorine crisis," which may be the first sign of improvement. The sulphates and the phosphates may be retained to a lesser degree, but do not return to normal at the same time as do the chlorides.

We have studied the records of 34 cases of acute lobar pneumonia in the Johns Hopkins Hospital, the total chloride output of whom was determined daily. Of these, 11 were on a pure milk diet, 1500 c.c. per day. Six terminated by crisis. In 2 the chlorides showed a drop toward the crisis, in 1 the crisis was preceded by a rise, while in other cases the rise began with, or even 4 or 5 days later than, the fall in temperature. It will be seen that in these very few cases we obtained very little evidence of prognostic value from the determination of the chlorides. In no case were the chlorides entirely absent. On the day before the crisis they varied from 0.7 to 2.1 gms., average 1.3 gms. The greatest rise began on the fifth day after the crisis, on which day it varied from 3.8 to 4.9 gms.

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[28] Ville and Moitessier Compt. rend. Soc. de Biol., liii, p. 673.

Of 22 cases terminating by lysis the daily output of chlorides in $\frac{7}{10}$ of the cases fell toward the lysis and in $\frac{3}{10}$ it began to rise 1 or 2 days before the temperature began to fall. On the first day of the lysis the output was above 1 gm. in 10 cases (averaging 26 gms.) and 9 gms. in 1 case. The chlorides were entirely absent in 3 cases before defervescence and in 2 cases during the fall of the temperature. In these cases therefore their entire absence was not a bad sign. They reached their lowest point during the fall of temperature in $\frac{1}{3}$ of the cases, just before the beginning of the lysis in $\frac{2}{3}$, and began to rise with the lysis in just $\frac{1}{2}$ of the cases. The chief rise began and was most rapid after the temperature had reached normal.

In 5 fatal cases the chlorides fell steadily until death in 3 cases, rose in 1, while in 1 case death was preceded by 6 days during which they were quite absent.

In 1 case of delayed resolution the chloride curve was very interesting. Nineteen determinations were made during a period of twenty-two days. The lowest amount was 4.3 gms. and this was after the lysis. For the most part it varied from about 5 to 10 gms. per day. In this case there was therefore comparatively little chloride retention.

In those cases in which the lysis or crisis is followed by several days of very slight fever the chlorides may not rise until the temperature is quite normal. In other cases with a normal temperature but with a continuous slight leucocytosis they did not rise until this had fallen below 10,000.

The chlorides of the urine are increased after chloroform inhalation, in diabetes insipidus and in other conditions with marked polyuria. They are decreased in all chronic diseases, in which cases the reason may be disturbed absorption, the diet, or the condition of the kidneys. They are decreased also in gastric diseases when there is considerable vomiting, when absorption is diminished as in malignant pyloric stricture, and when they are lost to the body by lavage or diarrhea.

It is an ominous sign in chronic diseases if the output of chlorides drops to as low as 2 gms. (provided the diet cannot explain this) for the cessation of chloride elimination sometimes is a sign of on-coming death.

The output is very low in meningitis and only moderately low in typhoid fever. It is markedly diminished in cholera, pyemia, puerperal fever, in serum disease[29] and in acute articular rheumatism. In cirrhosis of the liver it is said to be increased. The explanation by Widal of edema in nephritis as due to a specific retention of chlorides because of renal insufficiency, while the output of other solids remains normal, was accepted for several years and then abandoned. Those who adopted this theory applied the term "chloruremia" to a partial renal insufficiency for chlorine elimination, with a rapidly developing general edema, a low Cl output and an increasing output of albumin in the urine. It would be hard to explain on this basis the absence of edema after even a week of total suppression of the urine, *e.g.*, to calculus, or to the removal by operation of the only functioning kidney. We have repeated the work of Widal with varying success, but with no success at all if the water intake was controlled and this was difficult since the salt makes the patient very thirsty.

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[29] Rackemann, Longcope and Peters, Arch. of Int. Med., Oct., 1916, vol. xviii, p. 496.

ESTIMATION.—A rough estimation of the amount of chlorides in a specimen of urine is easily made by dropping 1 drop of $AgNO_3$ solution (1:18) into a test-tube of clear urine which contains no albumin and to which 10 drops of pure nitric acid had been added. If the chlorides are normal or increased in amount, the precipitate forms as a compact ball which sinks to the bottom; if diminished, this ball is less compact; if much diminished, *e.g.*, to 0.1% or less, only a cloud will be produced.

QUANTITATIVE DETERMINATION.—The best method of determining the chlorides of the urine is Arnold's modification of Volhardt's method. With the chlorides are estimated also the minute traces of cyanides present. The urine should contain no nitrites, and most observers add also, no albumin or albumose, since these will be precipitated as silver albuminates. If albumin be present, it may be necessary to ash the urine (Neubauer's method). Hammarsten recommends that the albumin be removed by heat and acetic acid, but the albumin precipitate must be washed for some time in order that the abundant chlorides which it will contain may be regained.

This method is as follows: The chlorides are precipitated by an excess of silver nitrate in a urine made strongly acid by nitric acid. The precipitate is then filtered out and the excess of silver nitrate determined by titration with ammonium sulphocyanate.

Solutions necessary: (1) An $AgNO_3$ solution 1 c.c. of which will exactly precipitate 10 mgms. of NaCl. The pure crystallized $AgNO_3$ is used, 29.075 gms. dissolved in 1 liter of distilled water.

(2) A cold saturated solution of iron ammonium alum, or ferric sulphate, chlorine free (50 gms. of Fe_2O_6 per liter).

(3) HNO_3, specific gravity 1.2, chlorine-free. If chlorine be present the acid should be purified by distillation. Any nitrous acid should be removed by the addition of urea.

(4) An ammonium sulphocyanate solution 10 c.c. of which will equal 10 c.c. of the silver nitrate solution. To obtain this, 12.9 gms. of the NH_4SCN are weighed and dissolved in a little less than 1 liter of water. Twenty cubic centimeters of the silver nitrate solution, 5 c.c. of the iron alum, and 4 c.c. of nitric acid are mixed in a flask and then diluted to 100 c.c. The ammonium sulphocyanate solution is then added from a buret. The precipitate, at first brown, at once changes to the white precipitate of silver cyanate, until the last particle of silver has been precipitated after which the brown ferric cyanate precipitate will persist. This end reaction is very sharp. The volume of the solution may then be corrected. Others recommend (v. Jaksch) that this solution be so made up that 25 c.c. of it will equal 10 c.c. of the silver nitrate, while others, that 20 c.c. equal 10 of the silver solution.

To determine its chlorides 10 c.c. of the urine are carefully measured with a pipet into a 100 c.c. measuring flask. Then are added 20 to 30 drops of nitric acid and 2 c.c. of the iron alum solution. If highly colored a few drops of 8% $KMnO_4$ also are added. The silver nitrate solution is then slowly run in from a buret, constantly shaking the flask until one is sure that all the chlorine has been precipitated and that there is an excess of the silver solution. It is usually safe to add 20 c.c. of the silver solution, while others recommend that 15 c.c. be used. In general the greater the excess the better the results. The flask is then allowed to stand for about 10 minutes, then filled to the 100 c.c. mark with water and thoroughly mixed. There should be an excess of iron present otherwise the nitric acid will decolorize the ferric cyanate but this excess of iron causes a brown rather than a red color in the end reaction.

The contents of the flask is then filtered through a dry filter until 50 c.c. of clear filtrate are obtained. This is titrated with the ammonium sulphocyanate solution until the end reaction. The amount used indicates the excess of the silver solution in 50 c.c. of filtrate. This amount multiplied by 2, since only ½ of the filtrate was used, and subtracted from the number of cubic centimeters of silver nitrate originally added, will give the number of cubic centimeters of silver nitrate actually precipitated by the chlorides of the urine. This multiplied by 10 mg. will give the weight of the chlorides as sodium chloride in the amount of urine used.

Some add the iron-alum solution to the 50 c.c. of filtrate, not before. A much-jaundiced urine should be decolorized by adding a few drops of potassium permanganate and nitric acid. The urine is then warmed, allowed to stand for a few minutes, and filtered.

Harvey's Method.—Harvey [30] recommends a modification of the Volhard method which seems accurate, and which certainly is much simpler.

The solutions used are the silver solution described above, a sulphocyanate solution prepared as above, 20 c.c. of which are equivalent to 10 c.c. of the solution of silver nitrate, and a third solution, the "acidified indicator," which is prepared as follows: To 30 c.c. of water are added 70 c.c. of nitric acid (sp. gr. 1.2, or 33%). One hundred grams of crystalline ferric ammonium sulphate are dissolved in this menstruum, and the solution is then filtered.

Five cubic centimeters of the urine are pipeted into a small beaker and diluted with about 20 c.c. of distilled water. The chlorides are now precipitated with exactly 10 c.c. of the solution of silver nitrate, and about 2 c.c. of the acidified indicator are added. The solution of ammonium sulphocyanate is then run in from a buret until the first trace of red persists throughout the mixture. The number of cubic centimeters of the sulphocyanate solution used is divided by two (since 20 c.c. of this solution equal 10 c.c. of the silver solution), and this quotient subtracted from 10.

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[30] Arch. of Int. Med., July 15, 1910, vol. 6, p. 12.

The difference is the amount of the silver nitrate solution used to precipitate the chlorides in the urine. Each c.c. of this is equivalent to 0.01 gm. of NaCl.

Phosphates.—The amount of phosphates weighed as P_2O_5 in the urine of an adult varies from 1 to 5 gms., average about 3.5 gms., in 24 hours. Of this the earthy phosphates are estimated as from 1 to 1.5 gms. and the alkaline from 2 to 4 gms. These form a constant sediment in an alkaline urine, a constituent of some of the most common crystals and the principal ingredient of some of the commonest stones. In addition to the mineral phosphates there is always in the urine a little phosphorus in organic combination. This amount in the urine depends especially on the amount in food and also inversely as the food's content of calcium and magnesium which form in the intestines insoluble phosphates, the most of which are eliminated with the stools. (Much of the acid calcium phosphate is absorbed from the bowel.) Because of this the phosphates of the urine may decrease to even less than 1 gm. in 24 hours. This is the reason why the urine of certain of the herbivora contains only traces of phosphoric acid. In metabolism experiments involving phosphoric acid one should control the diet carefully that the amount of this acid absorbed be approximately constant.

The phosphate output is increased by a nuclein-rich diet; by any condition which increases the metabolism of the body tissues (the amount from this source, however, is small); and by hard muscular work. In starvation it falls somewhat, yet less than the nitrogen. In dogs on a pure meat diet, $N : P_2O_5 : : 8.1 : 1$.

Clinically the phosphates of the urine have been the subject of much discussion. Some state that they are increased in extensive disease of bones, as rickets, osteomalacia, diffuse periostitis, etc.; in destructive disease of the lungs, especially in early tuberculosis; and in extensive disease of the nervous system. Nevertheless the evidence is not clear as regards any one of these diseases. In mental disease Folin and Shäfer [31] found that while during the periods of excitement the output of phosphoric acid might be relatively diminished, yet there was but little absolute change.

It would seem to be increased in meningitis, in yellow atrophy of the liver, in diabetes mellitus, in diabetes insipidus, after the use of chloral, of KBr, and lastly in phosphorus poisoning.

It has been found diminished; in pneumonia during the fastigium (in children, however, there may be a rise (v. Jaksch)) and increased with the crisis (but not always simultaneous with the rise of nitrogen and of chlorine; at this time the ratio between the earthy and the total phosphates may increase considerably. Since in tuberculosis these are said to be increased, Gouraud suggests this as of aid in the differential diagnosis between these two conditions); in typhoid fever (in 1 case the total P_2O_5 rose after defervescence from 1.5 to 13 gms.); in most chronic diseases, especially in renal disease (Purdy stated that in nephritis a diminution in the output of phosphate is almost as constant as is the albuminuria); in pregnancy, in which case it is attributed to the fetal bone formation; and in gout, in which disease the line of phosphoric acid elimination runs quite parallel to that of uric acid. And yet in all these diseases the output of phosphates is subject to large and sudden variations which are independent of the diet. Certain cases have been reported (Teissier) with all the symptoms of diabetes mellitus, except glycosuria, and a phosphate excretion reaching even 10 gms. in 24 hours, the so-called "phosphatic diabetes." This is a term applied by some to cases with a minimal output of the phosphates of at least from 3.5 to 4 gms. per day, but by others to cases in which $P_2O_5 : N : : 17–20 : 100$. Some say that these cases are merely neurasthenic; others that they are definitely diabetic with a rich phosphate output during the sugar-free periods.[32]

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[31] Am. Jour. of Physiol., vol. vii, p. 135.

The phosphorus which appears in the urine in organic combinations is apparently not influenced by a phosphorus rich diet, but would seem to be a good index of tissue catabolism (Mandel and Oertal).

In clinical chemistry one meets with 4 groups of phosphates—the diacid, monacid, normal and basic. These vary in solubility in the order in which they are named, the diacid being the most so. The monacid phosphates of calcium and magnesia explain the cloud which appears when urine is made alkaline by the addition of an alkali. The flocculent precipitate which appears when urine is heated and which resembles the cloud of albumin but is soluble in acetic acid, consists of normal calcium phosphate (basic, says v. Jaksch) with a trace of CaOx and $CaSO_4$, but no magnesium salts since its salts are more soluble than are those of calcium.

In leukemia, White and Hopkins[33] found the phosphate diminished both absolutely and relatively (to nitrogen) and suggest that the phosphorus is retained in the body to build new leucocytes. In the new-born the proportion between nitrogen and phosphoric acid is from 5 to 8 : 1.

Of the normal phosphates that of greatest interest is $MgNH_4PO_46H_2O$ of which the beautiful coffin-lid crystals of triple phosphate are composed (see page 248).

The acidity of the urine, due to many acid bodies and in an unknown degree to each, is, however, due chiefly to acid phosphates. Normally 60% of the phosphoric acid of perfectly fresh urine is present as diacid-phosphate and 40% as the monacid salts, but the former varies from 34.9 to 74.2%. In general it may be said that the urine will be amphoteric if the diacid salts are from 30 to 50% and the monacid from 70 to 50% of the total phosphate output.

An easy *approximate quantitative determination* of the phosphates of the urine is made by filling a test-tube half full of filtered urine, adding ammonia, warming and then allowing it to stand. If in from 18 to 24 hours the precipitate of the earthy phosphates is from ¼ to ½ an inch deep, the amount is normal; if less, it is diminished. This is then filtered out, all of the filtrate put back in this test-tube and 1 finger's breadth of magnesium mixture added. The urine is then warmed and the precipitate of alkaline phosphates allowed to settle. If during the same length of time the sediment is from ½ to ¾ inches deep the amount is normal.

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[32] Ralfe, Lancet, Mar. 5, 1887.

[33] Journal of Physiology, vol. xxiv, p. 42.

A urine may be cleared of phosphates by precipitating it with basic or neutral lead acetate.

Quantitative Determination; Uranium Nitrate Method.—Phosphoric acid is precipitated as a diacid salt by uranium nitrate. If cochineal is used as indicator the first excess of the uranium salt over and above that necessary to precipitate the phosphoric acid will give with the cochineal a green compound which serves as the end reaction. The uranium nitrate solution, although more stable than the acetate, should be frequently restandardized. Since the free nitric acid liberated in the reaction would redissolve a certain amount of uranium phosphate sodium acetate is added in excess; and in order that all phosphoric acid may be present as a diacid salt, acetic acid is added as well. The urine should be titrated while boiling since the end reaction will be quicker and sharper.

Albumin and sugar if present need not be removed. This titer changed with the volume of reagent used. For instance, if 20 c.c. are used, 1 c.c. will indicate 4.98 mgms. of P_2O_5; 21 c.c., 5 mgms.; 40 c.c., 5.14 mgms., etc. For this reason the uranium nitrate solution should be standardized against a phosphoric acid solution which has about the same concentration as normal urine.

The fluids necessary are: 1. A phosphate solution 50 c.c. of which contain 0.1 gm. of P_2O_5. This is so difficult to prepare that we recommend that it be purchased from those chemists who make a specialty of such work. This is the standard solution.

2. A solution containing 100 gms. of NaAc and 30 gms. of acetic acid, in 1 liter of water. Five cubic centimeters of this fluid added to 50 c.c. of urine will keep all the phosphates in the diacid condition and prevent the presence during the titration of any free nitric acid.

3. An alcohol-cochineal extract made by digesting the pulverized insects in 25% alcohol.

4. Uranium nitrate solution, 1 liter of which contains 35.461 gms. of $UO_2(NO_3)_2 6H_2O$. This solution is so standardized against solution 1 that 20 c.c. of this solution will equal exactly 50 c.c. of solution 1, that is, will indicate 0.1 gm. of P_2O_5. Three grams of NaAc are added since this salt always contains some free nitric acid.

To 50 c.c. of solution 1 in an Erlenmeyer flask are added 5 c.c. of solution 2, then a few drops too much rather than too little of the cochineal tincture. This fluid is then kept at the boiling point on a water bath or over a free flame, while the uranium solution is added from a buret in small amounts, shaking constantly. When near the end the precipitate which falls after each addition is allowed to settle somewhat and the bottom of the flask studied for the first trace of a green precipitate. Having determined how much of this solution will exactly precipitate the phosphoric acid in 50 c.c. of solution No. 1, the proper correction is then made and the result again verified that the result may be 20 c.c.

For the estimation of phosphoric acid in the urine 50 c.c. of this are treated in exactly the above manner.

If very accurate results are desired a table of corrections for the change in titer necessary for the volume used should be used.

If the urine is jaundiced or so colored that the end reaction is not sharp, it should be acidified with hydrochloric or nitric acid, decolorized with $KMnO_4$ and again neutralized.

Sulphates.—Sulphur is present in the urine in 3 forms—(*a*) preformed or neutral sulphates; (*b*) ethereal or conjugated sulphate, that is, sulphuric acid combined with aromatic alcohols, indoxyl, skatoxyl, cresol, phenol, etc.; (*c*) unoxidized, or organic sulphur. The normal person on a mixed diet eliminates from 1.5 to 3 gms., an average of 2.5 gms., of "*a*" and "*b*" (weighed as SO_3) in 24 hours. As a rule the ethereal sulphates make up about one-tenth of the total sulphates. Practically all of the sulphuric acid of the urine is a product of proteid metabolism, therefore its output runs parallel to that of nitrogen, and the ratio between them is quite, but not exactly, constant (100 : 19.1–20.4 Folin).

The total sulphate output is increased: in all conditions with increasing proteid oxidization, as a diet rich in meat; after exercise, providing this increases the nitrogen output as well; in fevers, since in this condition there is an increased proteid catabolism (especially in acute inflammatory disease of the brain and cord and in acute articular rheumatism); and after the ingestion of protoplasmic poisons. It is diminished during convalescence from an acute fever and in practically all chronic diseases. The amount of total sulphates has very little clinical value.

The ETHEREAL SULPHATES have attracted too much interest. While their output is subject to great and inexplicable variation, on the whole they are a fairly accurate index of the absorption of those products of intestinal decomposition which can pair with this acid. Among these are phenol, cresol, indoxyl, hydrochinin and pyrocatechin. While the most important of these are indoxyl and phenol yet these explain but about one-fifth of all the ethereal sulphate in the urine. Those which pair with the larger percentage of the sulphuric acid are still unidentified. It is the absolute not the relative amount of ethereal sulphates in the urine which is of value. Their output varies with the food. It is greatly increased by bad food and is diminished during periods of fasting. It is decreased by milk diet, since casein inhibits the growth of the bacteria of decomposition. Intestinal decomposition increases them both relatively and absolutely while calomel and similar drugs will at once decrease them. They are increased by the ingestion of aromatic bodies, especially of carbolic acid. There is almost none in the urine of the new-born. Their output is low when there is abundant hydrochloric acid in the gastric juice and is diminished by hydrochloric acid medication but increased by the ingestion of alkalies.

Pathologically, the ethereal sulphates are increased: in chronic colitis (and diminished in the acute), in constipation often but not always (in an interesting specimen of " black urine " from a case of extreme constipation the total sulphuric acid (as SO_3) was only 0.147 gm. per 100 c.c. of urine and of this 57% was ethereal sulphate; the following day the urine was of normal color and the total SO_3 was 0.086 gm. per 100 c.c. 50% of which was ethereal sulphate); in typhoid fever; intestinal tuberculosis; peritonitis; cholera (but during the stage of reaction little or none may be present); in atrophic liver cirrhosis and in carcinoma of the liver (in which cases the increase is attributed to the accompanying intestinal disturbance); and as a result of decomposition in other parts of the body than the intestine. It is of interest that in gastric disease, even in cases with much stagnation and fermentation, they are little affected.

The amount of UNOXIDIZED (*e.g.*, ORGANIC OR NEUTRAL) SULPHUR in the urine is supposed by some to vary with the amount and quality of the food; by others, to have relation not to food but to body tissue catabolism; to be increased by muscular work, by conditions leading to the oxygen starvation of the tissues and by the ingestion of various sulphur compounds, including the flower of sulphur, sulphonal, methylmerkaptan and ethyl sulphide.

The organic sulphur, which amounts to from 14 to 25% of the total sulphur, is present in an easily oxidizable fraction, which is oxidized by bromine or chlorine (bromine is better, since chlorine attacks also the taurin derivatives), and a difficultly oxidizable fraction.

To determine the total organic sulphur the dried residue after the removal of the sulphates must be fused with KNO_3. Fuming HNO_3 will not oxidize all of it. In cystinuria $HCl+KNO_3$ will oxidize only from 30 to 40% of it. One then determines it as sulphuric acid.

In jaundice from 24 to 60% of the sulphur in the urine is in unoxidized form and of this the difficultly oxidizable form is increased to about 4 to 5 times its normal proportion. In pneumonia it is increased and in liver disease, decreased. In cystinuria even 45.7% (in 1 of our cases 32 %) of the sulphur in the urine is in neutral form.

Edsall[34] who carefully studied the easily split (by alkali) sulphur in a series of cases decided that cystinuria is the only disease with an increase in the sulphur fraction and that the relative proportion of these 2 fractions has no clinical value.

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[34] Univ. of Penn. Med. Bull., 1892, iii, p. 87.

Recent work by several (*e.g.*, Benedikt)[35] has emphasized the independence between the neutral and total sulphur and the possible origin of the former in the catabolism of particular proteids.

Detection and Approximate Estimation.—In a test-tube of over 25 c.c. capacity is mixed the urine and about one-third its volume of an acid barium chloride solution ($BaCl_2$, 4; HCl, 1; H_2O, 16 parts). If the precipitate of neutral barium sulphate gives the urine a milky turbidity, the sulphates are normal; if creamy, increased; if merely a translucency, they are diminished. If the precipitate after settling from 18 to 24 hours fills ½ of the concavity of the tube the sulphates are normal. If this precipitate be removed by filtration, hydrochloric acid added to the filtrate and the fluid warmed the ethereal sulphates are split and precipitated as neutral sulphates.

Quantitative Determinations of Sulphur-containing Bodies.—The determinations of the sulphur bodies of the urine, the neutral, the ethereal sulphates and the total sulphur, are so important in metabolism experiments that the student should be trained in the general methods of this work. He should remember that these determinations, theoretically so easy and accurate, are in reality very difficult and so full of pitfalls that much preliminary practice should precede any important work. We have copied almost in full the method of Folin.[36]

Inorganic Sulphates.—About 100 c.c. of water (not less) 10 c.c. of dilute hydrochloric acid (1 part of concentrated HCl to 4 parts H_2O by volume) and 25 c.c. of urine are measured into an Erlenmeyer flask (capacity 200–250 c.c.). If the urine is dilute, 50 c.c. instead of 25, and a correspondingly smaller quantity of water may be taken. A 5% solution of barium chloride solution (10 c.c.) is then added, always drop by drop, preferably by means of an automatic dropper. The urine solution is not to be shaken, stirred or otherwise disturbed while the barium chloride is being added. At the end of an hour or later, according to convenience, the mixture is shaken up and filtered through a Gooch crucible. The precipitate is washed with about 250 c.c. of cold water, dried and ignited. Folin used porcelain crucibles rather than platinum and described the following technic: The asbestos for the mats must be good material, consisting chiefly of long shiny fibers. The fibers are cut with scissors into suitable lengths (50–70 mm.). A few grams at a time are then placed in a cylinder with about 300 c.c. of 5% hydrochloric acid and a strong air current is passed through for a few minutes. This separates all the fibers far more quickly and completely than the usual method of scraping them with a knife. In an hour or 2 asbestos enough for 200 crucibles can be prepared. It is kept ready for use in dilute hydrochloric acid. From 50 to 100 mgs. of asbestos are used for each mat. By using a good vacuum pump at almost full force the asbestos mat is packed into a thin but uniform and firm layer in the bottom of the crucible. It is then washed with the help of only enough of a vacuum to make the water run through in a slow stream; dried, ignited, and weighed. Mats so prepared are as effective as the best filter paper in retaining precipitates and there is practically no danger of losing any asbestos during the subsequent washings of precipitates of barium sulphate. The same mat can be used until about 1 gm. of barium sulphate has collected. Time is saved by not using the same mat too long because the filtration becomes slower and slower the more precipitate there is present and it is not safe to increase the vacuum too much.

The ignition of the precipitates is associated with more serious sources of error than the filtration, more serious because they are not accessible to direct observation.

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[35] Zeitsch. f. kl. Med., 1899, vol. xxxvi, p. 281.

[36] The Jour. of Biol. Chem., Jan. 1906, vol. i, p. 131.

The flame must not be applied directly to the perforated bottom of the crucibles. If this is done mechanical losses are sure to occur, even though the crucibles are covered with lids. Nor is it safe to apply the flame to sides of the crucibles. To do so involves again mechanical loss of barium sulphate. During the ignition the crucibles must be provided not only with lids, but also with tight bottoms. This is easily accomplished by the use of lids of ordinary platinum crucibles. The lid is placed on a triangle and the crucible stands in upright position on top, while the flame is applied to the platinum lid. Ten minutes ignition is sufficient unless organic matter is present.

TOTAL SULPHATES (Neutral, *i.e.*, Inorganic and Ethereal).—Of the following two methods Folin prefers the first: *a. Barium Sulphate Precipitation in the Cold.*—Twenty-five cubic centimeters of urine and 20 c.c. of dilute HCl (1 part HCl, sp. gr. 1.20, to 4 parts H_2O), or 50 c.c. of urine and 4 c.c. of concentrated hydrochloric acid are gently boiled in an Erlenmeyer flask (capacity 200–250 c.c.) for 20 to 30 minutes (not less than 20). To reduce the loss of steam it is better to keep the flask covered with a small watch glass during the boiling. The flask is cooled for 2 or 3 minutes in running water and the contents are diluted with cold water to about 150 c.c. To this solution is then added 5% barium chloride (10 c.c.) without any shaking or stirring during the addition. The remainder of the procedure is like that of the inorganic sulphate determination.

b. Barium Sulphate Precipitation in the Heat.—The boiling of the urine with hydrochloric acid is conducted exactly as in the preceding method. At the end of 20 to 30 minutes the boiling urine is diluted to about 150 c.c. with hot water. The mixture is heated once more to the boiling point, is then taken off the fire and at once precipitated with 10% barium chloride solution (5 c.c.). The barium chloride must always be added drop by drop. The filtration is made after about 2 hours' standing, when the mixture has acquired the room temperature. The remainder of the procedure is like that of the determination of inorganic sulphates.

ETHEREAL SULPHATES.—There is no need that these be separately determined since the difference between the amounts of total and neutral sulphates found in any given specimen will be an accurate index of the ethereal sulphate in that particular specimen.

TOTAL SULPHUR.—The determination of the total sulphur is one of the most important but most difficult problems of proteid metabolism. Folin's method is the following:

Twenty-five cubic centimeters of urine (or 50 c.c. if very dilute) are measured into a large nickel crucible (capacity 200–250 c.c.) and about 2 gms. of sodium peroxide are added. The mixture is evaporated to a syrupy consistency and is then carefully heated until it solidifies. This heating may seem a little slow, requiring about 15 minutes, but the conditions have purposely been selected to make it slow (by using as much as 3 gms. of Na_2O_2) in order to drive off as much ammonia as possible before the final fusion with more peroxide. The crucible is removed from the flame and allowed to cool. The residue is then moistened with 1 or 2 c.c. of water and, after about 7 gms. of sodium peroxide are sprinkled over the contents in the crucible, the mixture is heated to complete fusion for about 10 minutes. After cooling for a few minutes water is added to the contents in the crucible and the mixture is heated for at least half an hour with about 100 c.c. of water to dissolve the alkali and to decompose the sodium peroxide. The mixture is next rinsed into an Erlenmeyer flask (capacity 400–450 c.c.) by means of hot water and diluted to about 250 c.c. Concentrated hydrochloric acid is slowly added to the almost boiling solution until the nickelic oxide just dissolves (about 18 c.c. of acid to 8 gms. of peroxide). After a few minutes' boiling the solution should be perfectly clear. If it is not clear too much water or too little peroxide has been added for the final fusion. The insoluble residue must then be removed by filtration (after cooling) because it will not dissolve on the addition of more hydrochloric acid and too much acid must be avoided. The difficulty does not arise if little water and 7 or 8 gms. of peroxide are used.

To the clear acid solution are added 5 c.c. of very dilute alcohol (1 part alcohol to 4 parts H_2O) and the boiling is continued for a few minutes. The alcohol removes the last traces of chlorine which is always freed on acidifying the solution. Ten per cent. barium chloride solution (10 c.c.) is next added (by means of a dropper) and the solution left standing in the cold for *2 days* before filtering. The rest of the procedure is the same as for the other sulphate determinations.

Thiosulphuric Acid, $H_2S_2O_3$.—Normally there is either no thiosulphuric acid in the urine or not over 10 mg. in 1 liter. It has been found in some cases, as in typhoid fever.

Hydrogen Sulphide, H_2S.—This gas is seldom present in fresh urine. It does, however, occur and to it have been attributed cases of autointoxication. It was found in 1 case of long-standing eclamptic coma. It soon appears, however, in almost any urine on standing and may be detected by its odor, or by suspending in the mouth of the flask containing the urine a strip of paper moistened with sugar of lead solution plus 1 drop of NaOH. Air should then be blown through the urine. The paper will be blackened.

Sulphocyanic Acid, HSCN.—This acid occurs normally in the urine of man and of the animals which excrete nitrogen as urea, in amounts equalling about one-third that of the neutral sulphur. To demonstrate it, 100 c.c. of urine are precipitated by HNO_3, filtered, the precipitate washed, suspended in water, decomposed with H_2S and this filtrate distilled. The distillate, tested with Fe_2Cl_6, gives an intense blue color (Berlin blue) not modified by HCl.

Carbonates.—Carbonic acid is present in the urine, free, *i.e.*, which may be removed by a vacuum, and bound, in which case acid must be added to free it. Of the free, the urine contains about 180 c.c.; of the bound, from 2 to 10 c.c. The carbonic acid is increased by a diet rich in those organic acids which are oxidized to carbonates, hence there is much in the urine of the herbivora.

Silicic acid is present in traces as silicates. Its source is the food.

Nitric acid is present in all normal urines as nitrates. This is from the food.

Nitrous acid is often found in the urine as nitrates, but this was eliminated as nitrates and later reduced by bacteria.

Calcium and Magnesium.—The phosphates of the alkaline earths, calcium and magnesium, are present in the urine to the amount of about 1 gm. per day; calcium, weighed as CaO, about 0.12 to 0.25 gm.; and MgO from 0.18 to 0.28 gm. per day. Calcium, even that injected subcutaneously, is excreted chiefly through the intestinal wall and only about 4 to 29% through the kidneys, therefore the output in the urine is no index of the amount ingested which was used. Under normal conditions the most of the calcium eliminated is from the food. There is only a trace in the urine of persons on a vegetable diet. Its output in the urine runs parallel to that of ammonia and seems directly related to the excretion of acids. It seems to be increased by exercise. The most of that in the urine is eliminated in the morning, at which time the urine is most acid. It is increased both relatively and absolutely during periods of starvation when a slight acidosis is always present. This calcium is supposed to come from the bones. It can be decreased by the administration of alkalies.

The factors influencing the output of calcium in disease are but little understood. There is no increase in tuberculosis and none in rickets. The contradictory findings in the chronic diseases which have been the subject of so much careful study can best be attributed to the inanition which these diseases cause. In diabetes, and especially in the cases with acidosis, Gerhardt and Schlesinger found that the output in the urine is increased to even 2 to 4 times the normal amount, that it runs parallel to ammonia and can be diminished by alkaline treatment; that the normal ratio between the intestinal and the renal eliminations is reversed in favor of the latter and that there seems to be a retention of magnesium in the body. In cases of arteriosclerosis a retention of calcium has been demonstrated. In acute lobar pneumonia Peabody [37] found that calcium was retained but that the output of magnesium was normal or so increased that there is a definite loss to the body.

The relation of calcium to phosphaturia is interesting, since this metal would appear to be more to blame for this symptom-complex than is the phosphoric acid (see page 101).

Quantitative Determination of Calcium.—Of the filtered urine 200 c.c. are made alkaline with ammonia until a distinct precipitate is visible. This is then dissolved in the smallest possible amount of hydrochloric acid together with the addition of some NaAc. Ammonium oxalate is then added in excess and the fluid allowed to stand in the covered beaker on a water-bath for 12 hours. Bacterial fermentation should be prevented by the addition of thymol or carbolic acid. One thus avoids a precipitate of a calcium phosphate which would fall if the ammonia had not been added or if the urine becomes foul. After the 12 hours the supernatant fluid is decanted through a small ashless filter, the precipitate washed Cl-free by decantation with hot water and then finally brought onto the paper. The precipitate of CaO is very fine and apt to pass through the paper, hence is washed as much as possible by decanting. The washwater may be saved for the determination of magnesium. The filter paper is then dried and put into a platinum dish, burned slowly for a long time, then at a dull red till the mass on cooling is perfectly white. Since this ash will still contain some oxide of calcium it is moistened with a concentrated solution of ammonium carbonate, dried slowly and very gently ignited. This treatment with ammonium carbonate is repeated till constant weight as calcium carbonate is reached. One part of $CaCO_3$ equals 0.40 parts of Ca.

Another method is to burn the mass white with a blast-flame. The crucible is then cooled, weighed and the blast repeated until the weight is constant. The precipitate is now CaO, 1 part equalling 1.845 of calcium phosphate. Or, the precipitate is burned white and the concentrated ammonium sulphate then added and it is again burned and this repeated until there is no increase in weight. One part of the calcium sulphate ash equals 0.41176 part of CaO.

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[37] Jour. Exp. Med., January 1, 1913, vol. xvii, p. 71.

QUANTITATIVE DETERMINATION OF MAGNESIUM.—For determination of magnesium the filtrate and washwater of the calcium determination may be used. One-third volume of 10% NH_4OH (sp. gr. 0.96) is added which will precipitate all of the Mg as NH_4MgPO_4. This precipitate is allowed to settle well, is collected on an ashless filter, washed with water plus ⅓ volume of ammonia, dried thoroughly, shaken into a platinum crucible, the paper burned in a platinum spiral and its ash added to the crucible. The whole is then fused. Since the precipitate will contain some uric acid it will not burn quite white. It should, therefore, be cooled, a small piece of NH_4NO_3 and a few drops of water added, warmed slowly and then finally burned. The result is $Mg_2P_2O_7$. One hundred parts of this equal 36,208 parts of MgO.

Another good method is to treat 200 c.c. of the original urine in this way, which will determine the calcium and magnesium together. The calcium alone is then determined in a second portion. The difference will be the magnesium.

Sodium and Potassium.—The daily urine contains from 4.2 to 7.4 gms. of sodium weighed as Na_2O and from 2.3 to 3.9 gms. of potassium weighed as K_2O. The usual relation between them is 5 : 3.

The amount of these alkalies present will depend in general on the food. During hunger periods and also in fever the potassium may exceed the sodium but after the crisis the sodium will again predominate.

Severe exercise and also a vegetable diet will increase the amount of potassium.

Iron.—A trace of iron in organic combination is always present in the urine. It would be of great value could we determine this accurately, but unfortunately the methods used have too many sources of error. The reagents, for example, may contain more iron than the specimen to be examined. The amounts of urine-iron claimed as normal for 24 hours vary from 1 to 10 mg. It has been found increased in fevers, in malaria (even 16 mgms.) in pernicious anemia and in alcoholism. Neumann and Mayer,[38] using Neumann's method of ashing the urine, found that the daily output of iron of a normal person varied from 0.93 to 1.139 mgms. (average 0.983 mgm.). They found it increased especially in the urine of alcoholics and made the interesting observation that in diabetes mellitus the output of iron runs parallel to that of the sugar, being quite constantly 2.5 mgms. of iron per 100 gms. of sugar.

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[38] Zeitsch. f. physiol. Chem., 1902, vol. xxxvii, p. 2.

Lead.—To test urine for lead a considerable volume is evaporated to dryness and 50 c.c. of fuming HNO_3 added to the residue. After the reaction has subsided this is allowed to simmer over the free flame for ½ hour and 25 c.c. more of acid are added. This is repeated 3 times, each for 15 minutes. The resulting fluid is then evaporated to small volume, is neutralized with NaOH, filtered and is tested for lead with H_2S. If lead is present a brown precipitate will form.

Arsenic may be detected by saturating the faintly acid urine with H_2S, allowing it to stand for from 12 to 24 hours, then filtering, washing and treating the precipitate with bromine water, which will dissolve any arsenic sulphide. This solution is transferred to a suitable flask, zinc and sulphuric acid are added and the resulting stream of hydrogen is conducted into an acid $AgNO_3$ solution ($AgNO_3$ 0.1 to 0.2 gm.; HNO_3 2 gms.; water 10 c.c.). If AsH_3 is generated a blackish-brown precipitate of metallic arsenic is the result.

PIGMENTS OF THE URINE

Indoxyl Sulphate.—The value of the ethereal compounds of sulphuric, glycuronic and other acids, usually referred to as "indican," has been overestimated, yet they do have a certain clinical importance. Indoxyl sulphate, the chief, *i.e.*, the one of the "indican" bodies easiest to demonstrate, originates in the intestine as indol, a decomposition product of proteid. This is absorbed from the intestine, in the body is oxidized to indoxyl, is conjugated with sulphuric acid and is excreted as an alkaline salt. None is present in the urine of a new-born child or even before it is fed cow's milk. In adults on mixed diet a certain amount (from 5 to 25 mgms. in 24 hours), is always present. In man its output is greater on a flesh than on a vegetable diet. That present in the urine of a fasting person arises from the decomposition of the intestinal secretions. To be of value chemically this body must be present in the urine in considerable amounts. Often it does reach from 50 to 150 mgms. in 24 hours. It should at this point be emphasized that an increase of indoxyl and one of the ethereal sulphates are not synonymous. We usually estimate the amounts of both by the color tests of indoxyl but this may be greatly increased when the total ethereal sulphates are not and when the latter are increased only a relatively small amount may be bound to indoxyl.

In general the output of indoxyl sulphate (as indoxyl) is increased by the rapid decomposition of proteid either in the lumen of the intestine or elsewhere in the body. Its increase in cases of peritonitis and ileus is important, since its formation seems to depend upon the presence of trypsin. In paresis (from peritonitis or obstruction) of the small intestine the output of indoxyl shows a great and rapid increase; in paresis of the colon there is either no increase or one which begins late and is due perhaps to the bacterial decomposition of food. The determination of indoxyl would not help to differentiate between peritonitis and intestinal obstruction involving the same portion of the bowel. In one very interesting case of syphilitic stricture of the ileum the frequent attacks of partial obstruction due to the food could be accurately foretold by the increase of this body in the urine. It is much increased in cases of intussusception, of new growths and of twists of the small intestine. It is increased by intestinal putrefaction due to any cause, especially that present in the cholera infantum of children, in typhoid fever, in dilated stomach and some cases of nephritis. In these conditions a brisk purging will diminish its output greatly.

It is increased by the decomposition of albumin anywhere in the body; in gangrene of the lung, fetid empyema, putrid bronchitis (in which cases it may be present in very large amounts) and in advanced pulmonary or intestinal tuberculosis. Coriat thought its increase 1 element of the symptom-complex of akinetic mental conditions and its diminution 1 element of that of the hyperkinetic states. He considers this fluctuation in these patients not due to any intestinal condition nor to the diet. In certain cases of chronic constipation the urine may contain indoxyl in large quantities, but not necessarily much ethereal sulphates. One such case, a colleague of mine who enjoyed the best of health, furnished my classes for several years with urine very rich in this pigment. He gives a history of some severe abdominal condition when ten years of age, since which time he has been troubled with constipation.

The following is the analysis of his urine:

Total amount, in 24 hours, 1770 c.c. Color, clear yellow. On boiling, the color becomes dark brownish-red, almost black, with a dark magenta foam. Beautiful indoxyl test.

Total SO_3, 1.59 gms., of which the ethereal sulphates were only 14%. Total sulphur, 1.82 gms. (as SO_3) in 24 hours.

The urine gave a splendid indigo-blue but not Rosenbach's test.

It will be seen that despite the color on boiling and the good indoxyl test the ethereal sulphates of this specimen were not increased.

Its output is always diminished by closure of the pancreatic duct, but such closure cannot be assumed when there is absence of indoxyl sulphate unless other evidence is positive. It is increased when the HCl of the gastric juice is diminished. In nephritis it would seem to bear some relation to the albumin output but this needs confirmation.

Indigo calculi have been found.

The demonstration of indoxyl and the estimation of its amount depend on its oxidation to indigo-blue.

$$2C_8H_6NKSO_4+O_2=2C_8H_5NO+2KHSO_4$$

This reaction occurs if an oxidizing agent be added, or when the urine decomposes, in which case indigo may collect on the surface as a copper-red scum with a metallic glistening. Rarely, however, is enough present to be seen grossly.

Indigo-blue is a dark blue powder, insoluble in water, slightly so in chloroform but easily soluble in hot aniline. It is insoluble in alcohol and ether. It should be collected on an asbestos filter, washed with water then with alcohol (to separate the indigo-red) and then dried.

Indigo-blue may be sublimed at 300° C., giving off a purple-red vapor which cools in prismatic crystals of a copper-red, metallic color, but deep blue by transmitted light. If indigo-blue be mixed with hot alcohol and some very strong $NaOH$ and some glucose be added and this fluid fill a closed flask, indigo-white is formed. When this is exposed to the air indigo-blue will recrystallize out.

Tests of Indoxyl Sulphate—*Jaffé's Test.*—Albumin must be first removed from the urine by boiling and filtering. One test-tube is half filled with urine and another of the same size with the same amount of concentrated HCl. On the lips of this latter test-tube is placed the smallest possible drop of fresh concentrated $Ca(ClO)_2$. The HCl is then poured quickly into the tube containing the urine, carrying with it as it flows over the edge this drop of hypochlorite. The fluids are now mixed rapidly by inverting (not by shaking) the tube. One cubic centimeter or so of chloroform is then added to extract the indigo as it is formed. If necessary another drop of the $Ca(ClO)_2$ solution may be added after the fluids are mixed.

This test may be performed also as a contact test.

Hammersten advises to add to 20 c.c. of urine 2 or 3 c.c. of chloroform, an equal amount of hydrochloric acid, and then at once the $Ca(ClO)_2$ solution drop by drop, reversing the tube several times after each addition. The difficulty with this test is that a slight excess of the hypochlorite will destroy the indigo, giving yellow isatin.

$Ca(ClO)_2$ is a difficult substance to obtain pure, since it deteriorates so rapidly that the manufacturing chemists refuse to handle it. A pure salt is unnecessary, and the ordinary cheap bleaching powder or "chloride of lime" is satisfactory. (Chloride of lime is not calcium chloride, but a mixture of calcium hydroxide, calcium chloride, and calcium hypochlorite.)

Obermayer's Test.—The urine is first freed of disturbing substances by precipitating it with about one-fifth its volume of 20% $PbAc$, avoiding an excess, and then filtering. An equal amount of fuming hydrochloric acid containing a little Fe_2Cl_6 (4 parts of Fe_2Cl_6 in 1 liter of HCl) is then added. In a few minutes the blue color appears. The indigo can be extracted with chloroform.

If the urine contains potassium iodide a violet color results; if thymol, a bluish-green.

Since indol may disturb a bile test with HNO_3 all urines which contain indoxyl should first be extracted with acidulated chloroform before they are tested for bile.

Indigo if evaporated from chloroform will crystallize out in needles or plates.

Quantitative Determinations.—The quantitative determinations of indoxyl are in general unsatisfactory, and workers usually are satisfied with approximate estimations from the depth of color obtained by Obermayer's reagent. A more accurate result is obtained by repeatedly extracting the urine and evaporating the extracts in a weighed beaker. All indigo-red should first be removed by washing the residue with alcohol. The residue is then dried at from 105° to 110° C., and weighed.

Ellinger[39] found that but 85% of the amount of indigo theoretically present could be obtained in this way (evidently isatin is formed), and that neither the concentration nor the excess of reagent are of moment if the indigo-blue is extracted quickly enough.

Another method is to make the urine slightly acid if necessary with acetic acid, precipitate it with 1/10 its volume of PbAc and, if it is concentrated, dilute it ½ with water. To a measured portion of the filtrate is then added an equal volume of Obermayer's reagent. It is then shaken out several times with chloroform until this is no longer colored. (The amount of filtrate chosen should be such that 3 to 4 extractions for 2 minutes each time with 30 c.c. of chloroform are enough.) The filtered extract is distilled, the extract dried for 5 minutes then washed out 2 to 3 times with hot water (to remove isatin), dissolved in 10 c.c. of concentrated H_2SO_4, this solution diluted to 100 c.c. with water and titrated with a dilute $KMnO_4$ solution (5 c.c. of a 0.3% solution diluted to 200 c.c.) which has been standardized against pure indigo-blue. About 87% of the correct amount can in this way be measured hence in using this test a correction of 1/6 should be made. A double determination by this method requires about one and a half hours.

Strauss also used Obermayer's solution and extracted the indigo with chloroform, using a small separating funnel similar to that used in the lactic acid test (see Fig. 70). The combined chloroform extracts are measured, 2 c.c. are then removed and diluted till its color matches that of a standard tube of known content and from this can be reckoned the total amount.

Coriat proposed[40] a graduated separating funnel, in which the $Ca(ClO)_2$ test is made and the chloroform extract compared with a standard color.

Phenol is almost always increased in the urine when the output of indoxyl is, but the reverse is not true.

A urine rich in indoxyl is as a rule normal in color when voided. In certain cases, however, the oxidation has already occurred and the urine is green when voided (green because of the blue of the indigo and the yellow of the urine). Such cases have been described by Sahli, MacPhedran and Goldie, and by others, but must be excessively rare and methylene blue should always be excluded (see page 99).

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[39] Zeitschr. f. physiol. Chem., vol. xxxviii, p. 178.

[40] Am. Jour. Med. Sci., April, 1902.

Skatoxyl-Sulphate.—Skatol, another product of the bacterial decomposition of albumin, also is formed in the intestine and absorbed. By analogy we may suppose that like indol it is oxidized to skatoxyl, conjugated with sulphuric acid and eliminated by the urine. As a matter of fact, however skatoxyl sulphuric acid has never been actually demonstrated in the urine and the colors which would suggest its presence may as well be due to other red pigments.

The red or violet color produced by adding to the urine an oxidizing agent with a strong acid is usually attributed to skatol-red. With Fe_2Cl_6 it gives a violet color and with concentrated HCl it is decomposed, depositing a red precipitate. In Jaffé's test the urine becomes dark red or violet. On standing exposed to the air such urines darken from above downward, first red, then violet, finally even black. But as has already been mentioned, these colors are not conclusive for skatol-red. Rosin denies that skatoxyl has ever been proven present and thinks that all these tests could be explained by indigo-red. To prove that a pigment is skatoxyl it would be necessary to reduce it with zinc-dust and obtain skatol.

Indigo-red.—The pigment called indigo-red has several other names, *e.g.*, urorubin and urorhodin. This body is always formed with indigo-blue especially if Jaffé's test be made with warm urine. Indigo-blue and indigo-red are isomeres which arise from the same mother substance (indoxyl sulphate). Indigo-red forms spontaneously in decomposing urine and may form a sediment. Urorosein is formed at the same time.

Indigo-red crystallizes in dark reddish-brown or chocolate-brown needles or plates. Heated to 295° or 310° C. it sublimes with violet-red fumes. It is insoluble in water, dilute acids, and in alkalies. It gives a cherry-red solution with alcohol, ether, chloroform, and especially with glacial acetic acid. From dilute alcoholic solution it precipitates in crystals. From glacial acetic acid it is precipitated by soda or by water. It has a characteristic absorption spectrum.

Reduction Test.—The alcohol solution is made alkaline with sodium carbonate, a little glucose added and it is gently warmed. The solution decolorizes, but the color returns on shaking it in the air. This can be repeated as often as desired. If the pigment be boiled with even dilute caustic alkali the red is destroyed and various brown decomposition products result.

This pigment is present in large amounts in certain urines which give Rosenbach's test, but alone it is not responsible for the Burgundy-red color. It is increased especially in intestinal troubles; ileus, obstruction, cancer, etc. It is also present in large amounts in some urines which do not give a characteristic Rosenbach test, but these conditions are so various that they cannot be classified. It is found in traces in normal urine.

Demonstration.—Nitric acid added to a urine containing indigo-red gives a red color. A great deal of indigo-red is formed by Jaffé's test, especially if the urine be heated. After the urine is cold, it may be neutralized with soda and then shaken out with ether. The ether takes a fine red color and gives the absorption spectrum of this body. The ether extract may be evaporated in a watch glass and the crystals obtained.

Indigo-red is present in certain freshly voided urines, as in cases of pyelocystitis. It has also been found in concretions.

Among other red pigments of the urine is urorosein which is found in normal urine. This is characterized by its easy solubility in amyl alcohol and its insolubility in chloroform, ether and benzol. Ammonia and alkaline carbonates decolorize it at once while acid will restore the color. It has a characteristic spectrum. It is very unstable and decomposes rapidly.

To demonstrate urorosein one adds to the urine $\frac{1}{10}$ its volume of HCl and then filters. Urorosein makes a red stain on the filter paper. Urorosein also is produced by Jaffé's test, but is not extracted by the chloroform or ether.

The urine may contain other red pigments and after it is boiled with acid various brown pigments are produced to which beginners ascribe clinical value but which have none.

PARACRESOL AND PHENOLSULPHURIC ACID.—The daily urine contains from 17 to 51 mgms. of phenol and usually more paracresol. The sum of these 2 varies in various conditions and in general runs parallel to the output of indoxyl. The phenol is increased whenever indoxyl is, but the reverse is not always true. They are increased by a vegetable diet, in ileus and peritonitis, also in diphtheria, scarlet fever and erysipelas. Little is present in typhoid fever, smallpox and meningitis. They are products of decomposition which may take place anywhere in the body but especially in the intestine.

PYROCATECHIN (see page 98) is a similar body also eliminated conjugated with sulphuric acid, as also is

HYDROCHINON (see page 98) especially after carbolic acid poisoning.

POTASSIUM IODIDE of course often appears in the urine and must be excluded when testing for pigments. If HNO_3 and then chloroform are added to a urine containing KI the latter will take the pink color of iodine. Or, after the HNO_3, powdered starch may be added which will take a distinct blue color.

Bile Pigments in the Urine.—Bile pigment never appears in normal human urine (although it does in that of some animals). Bilirubin is derived from hemoglobin and so may be demonstrated in the plasma, and therefore in the urine, when there is increased breaking down of red blood-cells—*e.g.*, in the hemoglobinemia due to a blood poison. Bilirubin is very similar to hematin and is an isomer of hematoporphyrin.

The cases of jaundice have been divided into 2 groups; the "hepatogenous," due to complete obstruction of the bile passages, in which bile appears at once in the urine, as in cases of catarrhal jaundice, calculus in the common duct, cancer, or cirrhosis of the liver; and the "hematogenous" jaundice, formerly supposed to be the direct result of hemolysis by poisons, as the toxins of severe infections. In these cases bile will appear in the urine even before the skin or conjunctivæ are stained. Hematogenous jaundice was formerly explained as due to the inability of the liver to warehouse all of the free hemoglobin, but the correct explanation would seem to be that the bile capillaries are choked by the greatly increased amount of bile pigment which renders the bile too viscid to flow well through the bile ducts. "Toxemic jaundice" has been proposed as a better term for this condition.

The bile pigments and their derivatives of interest in clinical chemistry are bilirubin, biliverdin, bilifuscin, biliprasin, cholecyanin, and choletelin, all of which are products of bilirubin. Bilirubin is the only one which has been demonstrated in fresh urine. Biliverdin is often found in stale urine after the bacteria have had time to oxidize some of the bilirubin. The other derivatives explain some of the colors in Gmelin's test. It should be remembered, that all of the bile pigment present in a specimen of urine may be contained in the urate sediment.

BILIRUBIN, $C_{32}H_{36}N_4O_6$.—This is the one pigment of fresh human bile. It certainly can arise from hemoglobin elsewhere than in the liver. Its calcium salts occur in gall-stones; its crystals, the so-called hematodin crystals are met with in old blood extravasations. It is found in the fluid of certain cysts, especially of the breast and of the thyroid.

Bilirubin may be separated from mixtures of pigments by precipitating the fluid containing them with milk of lime in moderate amount while shaking well, saturating with CO_2 at once to prevent the decomposition of methemoglobin *et al*, and filtering. The precipitate is washed, dissolved in alcohol, chloroform is then added and then acetic acid to separate out the calcium. This fluid is filtered, the chloroform separated by adding water and the chloroform extract filtered through a dry paper and evaporated. The bilirubin of the residue is washed with a little alcohol and ether. The results by this method are always a little too low since calcium does not precipitate all the pigment and some is later destroyed. The work must be done rapidly. Hematin, if present, would also be isolated, but hematin does not occur in the body, except perhaps in the stomach and intestinal contents.

The crystals of bilirubin are rhombs, often with rounded edges, or needles which if pure have a beautiful brown-red color. They are perfectly insoluble in water, are soluble in alcohol and in chloroform, especially if hot, giving these solutions a brownish-red color. It may be precipitated unchanged from alcoholic solution by acids, it forms compounds with alkalies which are insoluble in chloroform but are soluble in water. (Hence bilirubin may be washed from a chloroform solution by an alkali. In this it differs from lutein.) It is precipitated by $BaSO_4$ and by $(NH_4)_2SO_4$.

An alkaline solution of bilirubin exposed to the air becomes oxidized to green biliverdin. In an alkaline (decomposing) urine, however, this seldom occurs since such would soon contain enough $(NH_4)_2S$, to change the biliverdin (and bilicyanin) back to bilirubin. Bilirubin itself may disappear from an alkaline urine, also from a urine preserved with chloroform. Bilirubin has no absorption spectrum.

BILIVERDIN, $C_{32}H_{36}N_4O_8$, occurs in the bile of many animals, but not in that of normal men. It is found, however, in the intestine and vomitus, and in jaundiced urine which has stood even for a short time. When pure, biliverdin is an amorphous, greenish-black powder, insoluble in water, ether or chloroform, but easily soluble in alcohol. In its insolubility in chloroform and its solubility in alcohol it differs from bilirubin. Its compounds with the alkalies are soluble to a green or a brownish-green solution. It is soluble in concentrated acetic acid and in HCl. The alkaline solution has no absorption spectrum, but an alcoholic weakly acid solution shows 1 band. With Gmelin's test it gives the same color changes as bilirubin. It can be reduced to bilirubin.

HYDROBILIRUBIN, $C_{32}H_{46}N_2O_7$, is considered by some to be the same as urobilin, but this is denied by the majority of workers. It is produced from bilirubin in the lower intestine by reducing bacteria.

BILIFUSCIN is a pigment which probably has not yet been isolated pure. The substance described ($C_{32}H_{40}N_4O_8$) is of an amorphous brown color, is soluble in alcohol to a deep brown solution, and in alkali, ammonia and dilute NaOH. It is insoluble in water and ether, and nearly soluble in chloroform. It is soluble in ether and chloroform if fatty acids are present. In the pure state it does not give Gmelin's reaction. Its spectrum is similar to that of biliprasin.

BILIPRASIN is said by some to be a mixture of bilirubin and bilifuscin, by others to be an intermediate stage between bilirubin and biliverdin, while still others consider it identical with biliverdin. The formula given is $C_{32}H_{44}N_4O_{12}$. Its alcoholic solution has no absorption spectrum, but the alkaline solution has. The brown color of its alcoholic alkaline solution is the chief point of difference between this and biliverdin. It differs from bilifuscin, since if acid be added to the above solution its color changes to green. The Gmelin test is of no value in recognizing this pigment.

CHOLECYANIN is a product of the oxidization of bile pigments with HNO_3, PbO, or $KMnO_4$. It may be further oxidized to choletelin. It gives a characteristic spectrum. Cholecyanin is insoluble in H_2O is soluble in alkalies and strong acids. It may be reduced to bilirubin. Its neutral or faintly acid solution has a bluish-green or steel-blue color with a beautiful red fluorescence. Its alkaline solution has a green color. The only way of recognizing it is by its beautiful spectrum.

CHOLETELIN.—Choletelin is a product of the oxidation of bilirubin with HNO_3. It is soluble in alcohol, giving a ruby-red solution. Its dilute solution has a yellowish-red color which does not change with change of reaction, as does that of urobilin. Chemically it is similar to urobilin, but its absorption spectrum differs, and with $ZnCl_2$ it gives no fluorescence. It is not precipitated by PbAc. It may be identified accurately only by the spectrum of its solution made acid with acetic acid. Its spectrum should not be confused with that of urobilin.

The reducible body of Stokvis is a by-product of the complete oxidation of bile pigment. It is a substance soluble in water, alcohol, alkali, and dilute acid, but not in ether or chloroform. It is not precipitated by PbAc, but is by PbAc and NH_4OH. Its characteristic reaction is, that if its alkaline solution be boiled with a reducing substance (*e.g.*, $(NH_4)_2S$) the solution becomes a beautiful rose-red color and gives an absorption spectrum. If this solution is shaken with air the rose red will disappear, but is soon restored with the disappearance of the spectrum.

The color of a bile-stained urine does not depend alone on the amount of bilirubin present, for a pale urine may contain much and a dark one, little. In general, the color of a jaundiced urine varies from a dark yellow to a brown or even to a greenish-black. An easy and accurate proof of the presence of bile is the yellow color of the foam when the urine is shaken since that of a very dark non-jaundiced urine will be pure white unless much urobilin be present. If a sediment fall in a bile-stained urine it will be yellow in color and may contain all the bilirubin of the specimen. Bilirubin will stain filter paper yellow. A jaundiced urine usually contains also an excess of urobilin and indoxyl, hence were the bilirubin to be removed its color would still remain dark. Such urine always contains the nucleo-albumin of bile. Heller's test for traces of albumin should not be applied to such urines since the oxidized pigment will confuse one.

Test.—If the urine contains much bile it may be shaken out with chloroform, the chloroform extract poured off and evaporated, the residue taken up again with chloroform and evaporated in a watch glass. Rhombic prisms of bilirubin separate out, which are soluble in alkali, which give Gmelin's tests and which on exposure to the air become green.

Gmelin's Test.—The urine in a pipet is superimposed over crude HNO_3 (sp. gr. at least 1.4) in a test-tube. (The HNO_3 used should be only faintly yellow with HNO_2. The amount of HNO_2 in the HNO_3 may be increased by adding a few pine shavings, or diminished by adding a little urea.)

If bilirubin be present strata of colors will be seen in the urine which from above downward are green, blue-violet, red, and just above the HNO_3, yellow. The green is the essential part of the test, not the other colors.

If too much HNO_2 is present the whole urine will soon be yellow. This test cannot be applied to very dark urines or to urines rich in indican, since the blue of the indigo and the yellow of the urine may give a deceptive green color, also the ring which forms at the point of puncture of the two fluids has a black tone and contains a fine precipitate. If in doubt the urine may be extracted with chloroform and both pigments tested for. A violet-red ring may be due to skatoxyl (?). The violet-red color also must, according to some, be present else the test might be confused with that of lutein, which gives a blue or a bluish-green ring, but in the case of urine this pigment will not confuse one. The violet-red color is due to skatol (?) and indoxyl. Biliverdin also gives this test. The test may fail if the HNO_3 contains too much HNO_2 since the green will not be seen.

Alcoholic solutions cannot be tested, since alcohol alone will give this test. If the urines to be tested are to be shaken out with ether, the ether must be alcohol-free, and this is not always the case.

If the urine contains much urobilin the bile test may be unsatisfactory, but this seldom happens. To avoid it the urine should be diluted to a specific gravity of 1.005, and then if the green color appears bilirubin certainly is present. Some always dilute the urine this much before making Gmelin's test since then only the green will appear.

The lutein of the serum does not disturb one much but methemoglobin may. If abundant albumin is present this should be precipitated and this precipitate extracted with chloroform since it will carry much bilirubin down with it. If but a trace of albumin and much bile are present the albumin may be disregarded, indeed it may even improve it, but if only a trace of bile and a trace of albumin are present the latter must be precipitated, dried and extracted.

The Gmelin test is said to indicate as little as 1 part of bilirubin in 80,000 of urine.

After the ingestion of antipyrin the test is said to be positive.

Rosenbach's test is the best modification of Gmelin's test. Indeed, it is far more sensitive than Gmelin's. As much as possible of the urine acidified with HCl is filtered several times through a filter paper on which will collect the bile-stained elements of the sediment and therefore the most of its bilirubin. The filter is then partially dried with dry filter paper and then 1 drop of yellow HNO_3 dropped upon it. Rings will be seen about the drop which will present the above-mentioned play of colors, the external one being green. If the paper has been allowed to dry, it should again be moistened with water dropping on the acid. Instead of filter paper Dragendorff uses a porous porcelain plate.

The following tests are excellent as a class exercise since they teach well the chemistry of bilirubin. A test-tube is filled full of urine, 2 c.c. of chloroform and 3 drops of HCl added and the whole thoroughly mixed. The bile pigment, which acts as acid, is set free from its alkali combination by the HCl and being more soluble in this condition in chloroform than in water, is extracted by the chloroform. The chloroform is then poured off into another test-tube and an equal amount of water added. One drop of NaOH will now transform the free pigment to an alkali salt, which is soluble in water. The aqueous solution may now be tested with nitric acid, or the chloroform extract may be evaporated in a watch glass and the crystals studied.

According to another method the urine is rendered alkaline with NaOH or soda and then precipitated with $BaCl_2$ or $CaCl_2$, or the hydroxides of these metals, as long as a colored precipitate falls. This yellow precipitate is then filtered off and boiled with alcohol plus a few drops of dilute H_2SO_4. If no pigment at all is present this fluid remains colorless; but if bilirubin is present a beautiful, clear, green solution is obtained. If the urine contained chrysophanic acid the fluid becomes orange-yellow. This test is positive when others are negative.

Bilirubin may also be extracted from acid urine with chloroform (add a few drops of HCl). To avoid an emulsion, however, do not shake too vigorously. In case it be necessary to test the urate sediment, and this may contain all of the bile pigment which was in the urine, the sediment is dissolved with soda and the solution tested for bilirubin.

Hammarsten's Test.—Hammarsten's reagent consists of 1 part of 25% HNO_3 and 19 parts of 25% HCl. This acid mixture is allowed to stand until it is yellow. Just before each test 4 parts of alcohol are added to 1 part of this reagent. If to a few cubic centimeters of this fluid just mixed are added a few drops of a pure bilirubin solution a beautiful permanent green color is at once obtained. By adding more acid we can get at will the other colors, even the yellow. This test is applied to the urine as follows: Ten cubic centimeters of the urine to be tested are poured into a 15 c.c. centrifuge tube, a few cubic centimeters of $BaCl_2$ or $CaCl_2$ solution added and the mixture centrifugalized for from ½ to 1 minute. The supernatant fluid is then poured off. To the sediment are then added 1.2 c.c. of the above acid reagent, the tube well shaken and centrifugalized for half a minute. If $CaCl_2$ were used, this last centrifugalization is not necessary. A green solution is obtained even if there is but the 1 part of bilirubin in 500,000 to 1,000,000 parts of urine.

Huppert's Test.—Ten cubic centimeters of urine are made alkaline with soda and $CaCl_2$ solution is added as long as a precipitate is formed. This is filtered through a small filter and the precipitate washed with water. The filter paper with the precipitate is then placed in a porcelain dish, acid alcohol (5 c.c. HCl in 100 c.c. of alcohol) added, and then heated. In the presence of bilirubin the color of this fluid becomes green or blue. This test is recommended in case the urine contains much indican or is quite dark in color.

Nakayama's Modification of Huppert's Test.—Nakayama's reagent consists of 95% alcohol 99 parts, fuming HCl 1 part and Fe_2Cl_6 4 gms. per liter of the above mixture. To 5 c.c. of the urine, acidified if necessary, is added an equal amount of 10% $BaCl_2$ solution and the fluid centrifugalized. The supernatant clear fluid is then poured off and 2 c.c. of the above reagent are added to the precipitate. The fluid is then heated to a boil.

A green or a bluish-green solution is obtained which on the addition of yellow HNO_3 becomes violet or red. The test is said to indicate 1 part of bilirubin in 1,200,000 parts of urine.

The following very important test together with its modifications has been reported under at least 4 different names, *Trousseau's*, perhaps, having priority. The urine, acidified if necessary with acetic acid, is mixed with a tincture of iodine; or a contact test made, the iodine tincture being superimposed upon the urine. Cl or Br also may be used instead of I. In the presence of bilirubin a fine emerald-green color is obtained which is not biliverdin but a substitution product of bilirubin with iodine. This test is more sensitive than Gmelin's; it may be made even more delicate if the tincture of iodine be diluted 1 : 10 with alcohol (hence a 1% iodine solution) and the urine be overlaid with this. A green ring which appears at once, or in 1 minute, will indicate bile (Rosin). Using this test there can be no confusion with indoxyl. It is said, however, that some normal urines will give a positive test.

Stokvis's Cholecyanin Test.—This test serves as a good control if bile be present together with other pigments in excess but it is not as delicate as are some of the above. To from 20 to 30 c.c. of urine are added 5 to 10 c.c. of ZnAc or of 20% $ZnCl_2$ solution. A little soda is added to reduce the acidity, after which it is filtered. The precipitate which contains all of the bile is now dissolved in NH_4OH. This solution of bile pigment now in the form of cholecyanin is next neutralized. It will have a blue-green color with a red fluorescence and will give a characteristic 3-band spectrum.

Some of these tests are good when bile alone is present, others in the presence of other pigments also.

Certain substances occasionally present in the urine after the use of rhubarb, senna and santonin should not be mistaken for bile since these urines become red on the addition of an alkali, but the original color is restored if the urine be again acidified.

It is important to recognize the crystals of bilirubin. They are often found in a jaundiced urine which has been concentrated for leucin and tyrosin. To demonstrate them the urine is rendered acid with HCl and allowed to stand in the cold. Bilirubin will precipitate out in intensely brown sheaths or rhombs often with rounded edges; their color should prevent any confusion.

It is sometimes desirable *to remove bile* from the urine. This may be done by extracting with chloroform the urine after it has been acidified with HCl or by boiling it briefly with a little animal charcoal. This latter method should be carefully used, since other substances, perhaps the one sought for, may also be removed.

$KMnO_4$ in acid solution destroys the bile pigments perfectly. For each 1 c.c. of urine are added 2 drops of HNO_3 (or of HCl) and 2 drops of 4% $KMnO_4$. The urine is then warmed and gently shaken.

Bouma[41] recommends the following *quantitative determination of bile:* To 10 c.c. of fresh urine are added 2 c.c. of 20% $CaCl_2$ solution. The urine is then almost neutralized with NH_4OH. The urine, still slightly acid, is then centrifugalized, the fluid decanted, the sediment shaken up with water and again centrifugalized to wash the sediment. The fluid is now entirely decanted and 5 c.c. of a mixture of 4 c.c. of absolute alcohol and 1 c.c. of Obermayer's reagent (1.5 gms. of Fe_2Cl_6 in 1 liter of HCl, sp. gr. 1.15) are added to the sediment. This is then poured into a test-tube and compared with a set of 6 standard tubes to match the biliverdin which has been formed. If much bilirubin (more than 100 mgms.) be present the urine is diluted with normal urine (thus not diluting the phosphates) and the determination repeated.

Melanin-Melanogen.—In the urine of patients with melanotic tumors melanin or melanogen (Mörner) may be observed. The chromogen is colorless but the urine on standing, or after the addition of an alkali or oxidizing agent, turns black, beginning at the top. This change of color may be intensified by adding HNO_3 or Fe_2Cl_6. This pigment is insoluble in chloroform, which prevents its confusion with indoxyl. It may settle out as an amorphous sediment. It is decolorized by boiling with HNO_3.

Rosenbach's Reaction.—If to certain urines, while boiling, strong nitric acid is added slowly drop by drop, their color will change to a Burgundy red and the foam takes on a bluish-red color. The color of the foam is the more important part of the test since the red of the urine may be due to urobilin. (If an excess of HNO_3 was used the color of the urine would be a yellowish-red or yellow and the foam yellow.) If soda or ammonia is next added drop by drop the result will be a bluish-red precipitate soluble in excess to a brownish-red solution. This test is said to be due to indigo-red (Rosin). It has the same significance as the indoxyl reaction.

Bile Acids.—Glycocholic and taurocholic acids are, contrary to a former belief, not present in normal urine, but they may be present in large amounts in the urine of patients with jaundice, especially of the obstructive type, although, even in these cases, the urine may contain almost none and in cases of toxic jaundice they appear but in traces. Their presence, however, has but little value in the differential diagnosis between obstructive and toxic jaundice. Their detection in the urine is impossible unless at least 0.5% is present.

To isolate the bile acids Thierfelder recommends that the urine be concentrated to a small volume, the residue extracted with strong alcohol and then filtered. The alcohol is then removed by evaporation and the residual fluid precipitated with basic PbAc and NH_4OH. This precipitate is brought upon a filter paper, is washed with water, dried, treated with boiling alcohol several times and filtered hot. To this filtrate are then added a few drops of soda solution to decompose the Pb salts. It is then evaporated to dryness, the residue extracted with absolute alcohol and filtered. To the filtrate is then added a great excess of ether. It is now allowed to stand in order that the sodium salts of the bile acids may precipitate out. These at first will be amorphous but later crystalline. The precipitate is dissolved in water and tested by Pettinkofer's test.

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[41] Deutsch. med. Wochenschr., 1904, No. 24.

Tyson Method.—From 180 to 240 c.c. of urine are evaporated to dryness on the water-bath, an excess of absolute alcohol added to the residue and this then filtered. To this filtrate are added from 12 to 14 volumes of ether which will precipitate the bile acids. This precipitate is then brought upon the filter paper, dissolved in distilled water and decolorized with animal charcoal

Pettinkofer's Reaction.—To a solution of bile acids in a test-tube are added a little cane-sugar and then slowly, drop by drop, concentrated H_2SO_4, shaking the fluid well all the time and keeping its temperature at about 70° C. by warming or cooling as the case may be. A precipitate of cholic acid forms first which soon redissolves. Then, as more H_2SO_4 is added, the color of the fluid becomes first cherry red and later a beautiful purple which in 8 days will change to a bluish-red. This play of colors is due to the reaction of cholic acid and the furfurol which is formed by the action of sulphuric acid on cane-sugar. The purple-red solution should be diluted with alcohol and examined spectroscopically. It has a characteristic absorption spectrum. This is necessary in order to exclude certain confusing substances which may be present, as albuminous bodies, many bodies easily decomposed by H_2SO_4, many pigments, amyl alcohol and oleic acid.

Udránzky's Test.—According to Udránzky's test, which is the best, 1 drop of a 0.1% watery furfurol solution is added to 1 c.c. of the solution to be tested. This is underlaid with 1 c.c. of concentrated H_2SO_4, the tube meanwhile being cooled to restrain the reaction. In the presence of but 0.033 mgm. of cholic acid one may get a positive test, *i.e.*, a red color, with a definite bluish tinge, which on standing becomes blood-red. If 0.05 mgm. is present the solution will give a distinct absorption line in the spectrum. The spectrum must always be examined for confirmation. A 10% cane-sugar solution will give as good a test as a 0.1% furfurol solution.

If cane-sugar is used in excess it will be burned, giving a brown or a black color. An excess of furfurol would give an orange color. Oxidizing bodies if present will prevent the reaction. Indoxyl gives a violet color. Since some concentrated normal urines give beautiful positive reactions, to avoid mistake the bile acids must be isolated as lead salts and then tested pure.

Hay's test for bile acids is certainly very easy and is said to be even more sensitive and accurate than Pettinkofer's test. On the surface of the urine (which has been cooled, if necessary, to a temperature not higher than 17° C.) is sprinkled a little finely powdered sulphur. If the sulphur sinks at once it indicates a bile acid content of 1 : 10,000. If it sinks after shaking gently and waiting for 1 minute, 1 : 40,000. It is positive if even but 1 : 120,000 of bile acid is present. The explanation given for this phenomenon is that bile salts lower the surface tension of the urine.[42]

Diazo Test.—Certain diazo bodies combined with aromatic compounds give a colored reaction. A urine test depending on this reaction is recommended by Ehrlich for clinical use. What body or bodies in the urine give it are unknown, but the empirical value of the test is beyond dispute if the technic of Ehrlich is accurately followed:

Fluids: (1) One-half per cent. $NaNO_2$. This should be quite fresh.

(2) Five parts of sulphanilic acid, 50 of HCl and 1000 of distilled water.

To 250 c.c. of the second reagent are added 5 c.c. of the first. Only a fresh mixture (not over 1 day old) should be used. Equal parts of the urine and this mixed reagent are shaken together until considerable foam is produced and ammonia is then quickly added in excess. Usually it is added drop by drop notwithstanding the warnings not to modify the original technic. If the test be positive the urine will take an intense red and the foam a more or less brilliant rose-red color. A brown color or a salmon tint are not positive. If the test is positive and the tube be allowed to stand a precipitate should form, the upper surface of which has a dark greenish-black, or violet, color. Some would have us wait 24 hours for this precipitate but most consider that the important part of the test is the rose-red color of the foam.

Since with strong enough reagents normal urines give a positive test Green recommends that 1 part of solution 1 and 100 of solution 2 be used. This renders the test more delicate and gives fewer unexpected positive results.

Zunz prefers the paramido-acetophenol of Friedenwald's formula[43] to the sulphanilic acid since the results are more delicate and intense (paramido-acetophenol, 50 gms., conc. HCl, 50 c.c., water, q.s. ad 1000 c.c.).

Four drops of a 0.5% solution of $NaNO_2$ and 10 c.c. of the above solution are mixed and shaken well with 10 c.c. of urine. About 3 c.c. of ammonia are now added and the color of the foam observed. Zunz adds the ammonia all at once, not drop by drop. He considers the color of the foam as the important, and the precipitate as the less important, part of the reaction. Disturbing bodies can for the most part be removed by shaking the urine out with amyl alcohol, and then removing by heating it on the water-bath.

The urine soon loses its property of giving a positive diazo test, but after a few days of ammoniacal fermentation the test may reappear.

If necessary to keep the urine several days before testing it, ether may be added.

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[42] Beddard and Pembrey, Brit. Med. Jour., March 22, 1902.

[43] New York Med. Jour., 1894, p. 745.

Some claim that to concentrate the urine on a water-bath to a syrup (Michaelis) will give a positive test although the fresh urine was negative. Zunz did the most of his careful work with such concentrated urines. That this does not always help matters was shown by Imhoff who found that some concentrated urine of rabbits with experimental tuberculosis gives a brown foam, but if diluted to its previous volume the foam is a brilliant red. Similar observations were made by Dr. Hirschfelder, who tested the undiluted and the diluted urine of patients as a routine. We have been in the habit of testing the fresh, the diluted and the concentrated urines. I am told that certain urines giving a negative test according to the usual technic will give a positive one if only ½ volume of reagent is used. Since a positive reaction may be due to impurities in the reagents (*e.g.*, in the ammonia) one should always control the test, using distilled water instead of urine.

What it is which gives the red color when combined with a diazo body is not known. Bondzinski found alloxyproteinic acid in all normal urines and since this will give a positive test he suggests it as the cause. Clemens, however, claims that the body giving the diazo test is sulphur-free.

OCCURRENCE.—The urine of normal persons never gives a positive test if dilute reagents are used. Ehrlich classified the diseases in which it may be positive into 4 groups: *a. Non-febrile diseases*, such as advanced heart disease, chronic hepatitis, carcinoma especially of the pylorus, leukemia, marasmus senilis, malarial cachexia, tuberculous abscess, etc. In all of these it is rarely positive.

b. Febrile Diseases.—(1) Those in which the test is almost never positive, *e.g.*, acute articular rheumatism and meningitis.

(2) Those in which it may or may not be positive, as pneumonia, scarlet fever, diphtheria, erysipelas and phthisis.

(3) Those in which it is very often positive, as typhoid fever and measles.

Lobligeois found the test positive in 42 of 52 cases of scarlet fever and in but 3 of 137 cases of diphtheria. It may therefore have some value in the diagnoses of cases of diphtheria with a scarlatinal rash. Brunschwig found that in children the reaction is always positive in typhoid, often in scarlet fever, quite often in measles, rarely in pneumonia and never in whooping-cough.

Ehrlich considered that in the first 2 groups of fevers a positive reaction means a bad prognosis; that its continued absence speaks strongly against the diagnosis of typhoid; and that its reappearance in a case of typhoid fever together with recrudescence of the fever speaks in favor of a relapse or recrudescence of the typhoid infection rather than to a non-typhoid complication or sequela. Long continued use has confirmed the accuracy of this test although it is not as important as formerly owing to the advances in bacteriology and serology. Its value in typhoid fever is also much lessened by the fact that it is usually negative in the early stage of the disease when it would be most helpful.

In phthisis a positive diazo test suggests a bad prognosis although the previous opinion of Michaelis that such cases were always fatal is not borne out by the experience of others. Boissière found it positive in 18 of 130 severe cases. There is some reason to think that in tuberculosis this reaction is due not to the tuberculosis but to a secondary infection.

It cannot be used to distinguish between typhoid fever and miliary tuberculosis since it is so often positive in both conditions. It is positive also in puerperal fever and in actinomycosis of the lung.

The work of Zunz [44] is of particular importance since it seems to have been done with exceptional care. His conclusions are that the test is valuable in the early diagnosis of typhoid fever and in the prognosis of tuberculous pneumonia, although in the latter disease a positive reaction does not necessarily mean a hopeless prognosis; that it helps in the early diagnosis of measles; that it is in favor of tuberculosis in cases of peritonitis, pleurisy, and nephritis; that it is often present in erysipelas; that if present, the prognosis in a case of cancer or sarcoma is immediately grave; that in cases of pneumonia and pyothorax (non-tuberculous) the test means merely a disturbed metabolism; that in certain cardiac affections it speaks in favor of a reserved prognosis; and, in conclusion, that it is a useful test, although its value has been much exaggerated.

Many consider that the ingestion of certain drugs inhibits the test, as, for instance, phenol, salol, benzonaphthol, etc.

Plezl [45] found it positive in typhoid from the middle of the first to the end of the third week and in measles before and during the period of eruption. His suggestion is that apart from these conditions it is positive in streptococcus septicemia, which also explains its presence in the angina of scarlet fever, in advanced phthisis and other forms of severe tuberculosis.

We have found the test very valuable, expecially in the diagnosis of typhoid fever. In our typhoid cases, however, the test very soon becomes negative, evidently the result of the diuresis we encourage.

For a discussion of *Ehrlich's dimethylamidobenzaldehyde reaction* the reader is referred to Simon's article [46] and for *Rosso's test for typhoid fever* to the discussion by Neuman and Behrend.[47]

FERMENTS

Several ferments have been demonstrated in the urine in health and in disease and in amounts which depend on the general condition of the patient. The most important of these is **pepsin.** To demonstrate this a mass of pure fibrin is covered for several hours with the fresh urine. This will absorb a great deal of the pepsin. The fibrin is then removed from the urine and dropped into a flask containing dilute HCl, and placed in a thermostat. If the fibrin becomes digested pepsin was present. Trypsin, it is said, has been found in the urine, but this has not yet been confirmed. A **diastatic ferment** has been demonstrated in some urines and the same is true of rennin. It is claimed that the decomposition of urea with the formation of ammonia bodies is due to a ferment rather than to bacteria but this has never been proven.

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[44] Bull. de l'Acad. roy. de méd. de Belgique, ser. iv., t. xiv., p. 553.

[45] Wien. klin. Wochenschr., 1903, No. 31.

[46] Am. Jour. Med. Sci., 1903.

[47] Arch. of Int. Med., April 15, 1913, vol. xi, p. 456.

If **lipase** [48] is present at all in normal urine it is only in traces. It is found in jaundice, perhaps in diabetes mellitus, but especially in those conditions accompanied by fat necrosis (in dogs after mechanical injury of the pancreas and after tying the pancreatic duct), and in tuberculosis during the fibrile periods.[49]

METHOD (KASTLE-LOEWENHART).—Into each of 3 flasks are measured 5 c.c. of urine. The second flask is then boiled. To the urine in the third are added 3 drops of phenolphthalein (1%) and then *0.1N* NaOH till faintly pink. This amount of alkali, determined by this titration, is then added to flasks 1 and 2. To each of the flasks are then added 0.25 c.c. of ethylbutyrate and 0.1 c.c. toluene and they are left in a thermostat at 39° C. for 20 hours. At the end of this period an amount of *0.1N* HCl which is 0.5 c.c. more than the amount of *0.1N* NaOH previously added, is measured into each, each flask is then shaken out with 50 c.c. of ether and 25 c.c. of alcohol, 3 drops of the phenolphthalein solution are added to the ether extract and the amount of butyric acid split off titrated with *0.1N* KOH.

In case 5 c.c. of urine are not available for each flask the figure obtained from the smaller amount is calculated for 5 c.c. using the formula that the amount of ferment action varies as the square root of the amount of ferment present.

Amylase of the Urine.—Wohlgemuth's method for the quantitative estimation of amylase in the body fluids is as follows: [50]

Ten carefully cleaned and dried test-tubes numbered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 are placed in a test-tube stand and to each tube except Tube 1, which contains 1 c.c. of the fluid to be tested, is added 1 c.c. of a normal saline solution. With a 1 c.c. pipet, accurately graduated to 0.1 c.c., 1 c.c. of fluid to be tested is added to Tube 2, thoroughly mixed drawing up and expelling 3 times the mixture of normal saline solution and test fluid. Then finally draw up 1 c.c. of the fluid now diluted 1 to 2 and place this in Tube 3, repeating the same process of mixing and again withdrawing 1 c.c. of mixture. This is to be repeated in all the succeeding tubes and in the last tube the 1 c.c. withdrawn can be discarded. This will give the following amounts of the fluid to be tested in each tube (7 tubes suffice in most cases).

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[48] See Hewlett, Jour. Med. Research, 1904, vol. vi., p. 377; also Garnier, Compt. rend., 1903, vol. v, p. 1064.

[49] White and Zeedick, M. of the Assoc. of Am. Phys., 1916.

[50] Geyelin, Arch. of Int. Med., 1914, xiii, p. 96.

No. 1 = 1.0 c.c.; No. 2 = 0.5 c.c.; No. 3 = 0.25 c.c.; No. 4 = 0.125 c.c.; No. 5 = 0.0625 c.c.; No. 6 = 0.032 c.c.; No. 7 = 0.016 c.c.; No. 8 = 0.008 c.c.; No. 9 = 0.004 c.c.; No. 10 = 0.002 c.c.

Now to each tube are added 2 c.c. of 0.1% starch solution (Kahlbaum's soluble starch). The tubes are shaken gently and fitted into a wire cage which is placed on a water-bath at 38 C. for ½ hour. The temperature should be kept within ½ degree of 38 C. and the time accurately measured. At the end of this time all tubes are withdrawn and placed in ice-water for 5 minutes to inhibit further amylolytic activity and are then replaced in test-tube stand. To each tube is then added 2 drops of *0.025N* iodine solution which must be freshly prepared daily from a stock solution of *0.1N* strength. One-to-one-thousand starch solution should be freshly prepared every 3 days and kept in the refrigerator.

If the urine is normal the first 2 or 3 tubes will be golden yellow in color, all starch having been digested, while the fourth will be reddish with no tinge of blue, the fifth violet and the others blue. The tube from which the reading is taken is the deep-red tube. This color shows that all starch has been digested at least to the dextrin stage.

The calculation is then made as follows: In Tube 4, *e.g.*, there was 0.125 c.c. urine or fluid which digested 2 c.c. of a 0.1% starch solution in ½ hour at 38 C. Therefore 1 c.c. of fluid to be tested would digest starch according to the following equation:

$$0.125 : 2 \text{ c.c. (starch)} : : 1 \text{ c.c.} : X = 16.$$

One cubic centimeter of fluid digested 16 c.c. of a 0.1% starch solution in ½ hour; this Wohlgemuth designates as follows:

$$d\frac{38^\circ}{30'} = 16$$

When 24-hour digestion is employed he expresses it

$$D\frac{38^\circ}{24 \text{ hr.}} = X.$$

The conclusions thus far obtained with this test are interesting although not yet valuable. Under normal conditions the d (units of amylase per 1 c.c. of urine) is almost constant, the lower normal limit being 8, but in 77.1% of the cases of nephritis is sub-normal or zero. In the other cases the urine contained considerable albumin which itself increases enzymatic activity or is accompanied in its excretion by the excretion of more amylase. The d values run fairly parallel with the elimination of phenolsulphonephthalein but in cases of cardiac decomposition the latter usually is subnormal but d normal.

CARBOHYDRATES AND ALLIED BODIES IN THE URINE

Certain carbohydrates in small amount are normal ingredients of the urine. Three such have been demonstrated; glucose, animal gum and isomaltose. Bodies related to carbohydrates also may be present in normal urine; *e.g.*, the paired glycuronic acid compounds, chondroidin-sulphuric acid, nucleinic acid, the mucoid of the nubecula and, sometimes, pentose. The total output of all of these carbohydrates measured as glucose amounts to from 2 to 2.23 gms. in 24 hours. Of glucose itself the normal urine may contain from 0.38 to 0.62 gm. in 24 hours (Naunyn, 0.4 to 1.4 gms.). The latest report is that of Myers [51] who concluded that the urine of normal persons may contain from 0.08 to 0.2% of sugar.

The total carbohydrates of the urine, both the fermentable and the unfermentable, may be determined as benzoylesters. The urine is made alkaline with NaOH and the phosphates removed by filtration. To the filtrate in a flask is added benzoylchloride, 4 c.c. per 100 c.c. of urine, and 40 c.c. of 10% NaOH. The flask is now shaken gently for 10 minutes (to avoid emulsion), then vigorously for 20 to 25 minutes, until all odor of the benzoylchloride has disappeared. It is then allowed to stand for a few hours, but not over night since the precipitate would get sticky and not filter well, then filtered, the precipitate washed, dried over H_2SO_4 and weighed.

While a normal person should be able to digest, warehouse and use an indefinite amount of starch ingested in 1 meal without the appearance of sugar in the urine, yet in the case of sugar there is for every person a limit to the amount which can be thus ingested without producing a glycuresis. The largest amount of glucose which a person can ingest without glycuresis is called his assimilation limit. For the normal person this is about 200 gms. (some say 300 gms.). Any amount beyond this produces a slight glycosuria of seldom more than 1 gram of glucose. Such a glycosuria is termed an *alimentary glycosuria*, or an alimentary glycosuria e saccharo. This follows directly the ingestion of the sugar and lasts but a few hours. A glycosuria which follows a meal rich in starch is called an alimentary glycosuria e amylo. This indicates a worse injury to the carbohydrate metabolism than does a glycosuria of the e saccharo group, and is practically always diabetic. There are on the other hand conditions (*e.g.*, pituitary disease) which raise the assimilation limit so that a person may consume even 500 or more grams of glucose and remain sugar-free. For each sugar there is a different assimilation limit. Hofmeister found that galactose and lactose passed the most readily into the urine, while for dextrose, levulose and cane sugar the limit is much higher.

A *spontaneous glycosuria* is one which appears while the patient is on an ordinary mixed diet; that is, a diet which does not contain an unusual amount of carbohydrate. A temporary spontaneous glycosuria is one which lasts but a few days. A spontaneous glycosuria which continues for several weeks or longer is usually diabetic.

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[51] Proc. Soc. Exp. Biol. and Med., 1916, xiii, p. 178.

An alimentary glycosuria occurs more readily if the sugar is eaten while the stomach is empty. Hunger lowers the assimilation limit considerably; pregnancy also does the same. Diseases which may lower the assimilation limit are: cirrhosis of the liver, cerebral disease, poor nutrition, fatty liver, phosphorus poisoning, the infectious diseases, certain neuroses, exophthalmic goiter, and any condition causing diuresis. Among the diseases which raise the assimilation limit are myxedema and pituitary gland disturbances. While most normal persons have an assimilation limit for glucose of 200 gms. or over, yet this varies much and 100 gms. has been accepted as the lowest limit of normal.

The following *method of determining the assimilation limit* was recommended by Naunyn. Two hours after a breakfast consisting of coffee with milk (about 250 c.c.) and 80 to 100 gms. of bread, the patient ingests 100 gms. of dextrose. If a definite glycosuria results the assimilation limit of that patient is pathologically lowered, while if the glycosuria is 1% or over one has very good reasons for suspecting diabetes mellitus. The glycosuria begins in about 1 hour after the meal of glucose, reaches its maximum in from 2 to 4 hours and lasts at the longest but 8 to 10 hours. The assimilation limit for cane-sugar is practically the same as that for glucose (from 150 to 200 gms.). Some prefer to use this sugar rather than glucose.

The reasons why the breakfast precedes the test meal is that if the latter is received into an empty stomach the sugar may pass too rapidly to the bowel and escape digestion, and that some which reaches the lower part of the small intestine may be absorbed by the lymphatics rather than by the portal vessels and so not pass through the liver but at once enter the systemic circulation and so be excreted.

That all glycosuria is not diabetic or that the prognosis of diabetes is not as dark as is usually believed is indicated by the observations of Barringer and Roper [52] who examined by repeated assimilation tests the condition of a series of patients in whom 5 years before a transitory spontaneous glycosuria had been discovered. Of these, 20% had during this interval become definitely diabetic; 15% had probably, but not certainly, become diabetic; in the case of 10% there was much doubt as to whether their condition was diabetic or not; while 55% were surely not diabetic. These writers, therefore, refuse to agree with Von Noorden that all persons who show even occasionally a spontaneous glycosuria are of necessity cases of latent diabetes.

To determine whether a transitory spontaneous glycosuria is diabetic or non-diabetic the patient's assimilation limit is tested a few months after the glycosuria has disappeared and again a few months later.

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[52] Am. J. Med. Sc., June, 1907.

The improved methods of determining blood-sugar have recently made it possible to measure carbohydrate tolerance by the study of the blood rather than of the urine after a test meal (see page 552). This has 1 definite advantage since the variable threshold limit for sugar is eliminated.

The Hunger Diabetes of Hofmeister.—Hofmeister found that if dogs under close confinement are kept on a poor diet, yet not starved, a certain number of them soon became diabetic and excrete 30% of the starch of their food as sugar. Naunyn believed that this is probably the explanation of some cases of that glycosuria in men which develop in cachexia producing diseases. Later Hoppe-Seyler reported [53] 10 cases of temporary glycosuria in vagabonds whose lives had been unhygienic and whose diet had been miserable. Their glycosuria disappeared in 24 hours after their physical condition had improved somewhat.

Glycosuria.—That a trace of glucose is present in normal urine can be proved by isolating glucosozone from large amounts of urine. Quantitative reduction tests before and after the fermentation of a normal urine also indicate the presence of this body. That is, a glycosuria is both normal and constant.

When the output of glucose in the urine is sufficient in amount that the glucose may be detected by the tests in common clinical use the condition is termed a pathological glycosuria or a glycuresis. Theoretically, a glycuresis will develop: (1) When there is a hyperglycemia of about 0.3% or over (see page 550). Such a hyperglycemia may be due to the ingestion of more sugar than can be warehoused or to the accumulation in the blood of glucose which the body cannot use and which therefore the kidneys will excrete. (2) When the ability of the kindeys to retain glucose is diminished, *e.g.*, after the injection of phlorizin and in the interesting condition called renal diabetes. (3) When the glucose exists in the blood in some chemical combination which renders it unfit for use.

A diabetic glycosuria differs from other glycosurias, according to Allen, in one fundamental particular: In the normal individual the greater the amount of sugar ingested the more is used, while in the diabetic individual the reverse is true (Allen's paradoxical law). That is, the limit of tolerance in the diabetic individual is real, not apparent as in the normal individual, and if these limits are exceeded by the ingestion of more sugar than the diabetic individual can handle, his tolerance is at once lessened rather than increased as in the case of the normal individual. According to Allen, the diabetic condition is one in which there is a deficiency of pancreatic amboceptor, by which term he designates a substance furnished by the pancreas which unites with glucose and forms a combination which the tissues can use. Glucose not in this combination circulates as a crystalloid which cannot be used by the cells, which acts as a diuretic and which is eliminated unchanged. In his earlier work Allen considered the problem of diabetic glycosuria as essentially one of functional pancreatic fatigue and indeed in many ways this would describe the condition well.

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[53] Münch. med. Wochenschr., April, 1900.

Clinically, cases of glycosuria may be classified as follows:

1. The group of diabetic patients who have a definite limit to their glucose tolerance. In these cases one assumes always a disease of the pancreas. In dogs whose pancreas has been destroyed or removed the glycosuria may reach 10 or 22% and the animal lives not over 4 to 5 weeks. In these dogs practically all the sugar ingested is excreted in the urine and in an amount which bears a quite constant ratio to the urine nitrogen (2.8 : 1). In no cases in man is quite all the glucose excreted and all patients have some tolerance to sugar. In some patients is found atrophy of the pancreas as a whole or, as in Opie's case, a degeneration limited to the islands of Langerhans. A very good illustration is reported by Mosenthal.[54]

2. Glycosuria may follow oxygen starvation of the tissues due to many causes; suffocation and the death agony; certain poisons, as CO, curare and amyl nitrite; narcotics, as ether or chloroform.[55] The work of King, Mayle and Haupt,[56] however, throws much doubt on the belief that the glycosuria of ether anesthesia is due to oxygen starvation.

3. Certain poisons, including morphia, strychnine, cocaine,[57] fusel oil, $HgCl_2$, and certain acids can cause glycosuria. In this connection the work of Herter [58] is interesting. He showed that the local application to the pancreas of reducing substances (adrenal extract and various poisons, H_2S, KCN, H_2SO_4, etc.) may cause glycosuria.

4. Severe cooling of the body.

5. The use of caffeine, theobromine, or any diuretic which stimulates the kidney may cause glycosuria (renal diabetes). This and phloridzin diabetes are the only cases of glycosuria which are not associated with hyperglycemia. Some patients with chronic nephritis have diabetes also, but, as a rule, the diabetes was their primary trouble. There is a tendency for the glycosuria of a case of nephritis to lessen because of an increased sugar tolerance as the nephritis progresses. This explains an old belief that Bright's disease cures diabetes.

Glycosuria sometimes follows a transfusion with normal salt solution, the injection of sugar into the blood, insults and injuries to the liver (well seen in animal experiments), cirrhosis of the liver and diseases and injuries of the central nervous system, the best illustration of which in animals is the piqûre of Claude and Bernard which causes an hyperglycemia of even 0.7%, which may last from 6 to 48 hours, and a glycosuria which in rabbits may reach even 6%. In man a transitory glycosuria which may be somewhat similar in nature sometimes follows apoplexy. This begins, as a rule, about 2 hours after the hemorrhage, lasts even 6 days, and may reach even 1 or 2%. Glycosuria may also be associated with brain tumors, especially those of the base, often with dementia paralytica, epidemic cerebrospinal meningitis, tabes, multiple sclerosis, diseases of the sympathetic nervous system and severe trauma of the skull. This last group is interesting. The glycosuria may begin at once or even a year later and usually is permanent. Such cases as a rule are mild. In some one may suspect an interesting relation of diabetes insipidus since they begin as an intense sugar-free polyuria and later develop a glycosuria. Glycosuria is met with also in some patients with functional (?) neuroses, psychical disorders, in exophthalmic goiter, gout, arteriosclerosis and obesity.

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[54] Arch. Int. Med., March 15, 1912.

[55] See also Brown, Johns Hopkins Hosp. Bull., May, 1900.

[56] Jour. of Exp. Med., August 1, 1912, vol. xvi, p. 178.

[57] See also Neubauer and Vogel, p. 92.

[58] Am. Med., 1902, p. 771.

In 90% of the severe grades of hyperthyroidism there is present an hyperglycemia and in almost as many cases a glycosuria, either spontaneous or alimentary. In the milder cases an alimentary glycosuria and hyperglycemia can usually be easily produced (2 hours after 100 gms. of glucose) and the return to fasting blood-sugar conditions is slower than normal.

A hyperglycemia and often a spontaneous glycosuria can be obtained in hyperthyroidism following the subcutaneous injection of adrenalin (Goetch's test, see page 552).

Emotional glycosuria. Folin, Denis and Smillie,[59] following the work of Cannon, Sbohl and Wright on animals, found glucose in the urine of 22 of 192 patients suffering especially from depression, apprehension or excitement, and in that of 58 of 664 patients at another hospital for the insane. They then examined students just before and after hard examinations and found after the examinations sugar in the urine of 18% of the men and of 17% of the women, all of whom had been sugar-free before the mental strain. They therefore consider that pronounced mental and emotional strain may produce temporary glycosuria in man.

Qualitative Tests for Glucose.—Since the most popular tests for glucose make use of copper solutions the students should study carefully the several reactions involved in the reduction of copper by a urine containing glucose, and, whatever the copper test he may later choose as his routine method he should first study Trommer's test, since in this each step in the reaction is taken separately and the chances of error are all very apparent.

Trommer's Test.—To a test-tube half full of urine is added about ⅓ its volume of 10% NaOH or KOH and then a 10% solution of $CuSO_4$ in drops, until a few flakes of the $Cu(OH)_2$ which form do not disappear on slight shaking. The upper layer of the urine is then warmed. If sugar is present in pathological amounts a precipitate, yellow or red in color, appears at once at the top. The heating should then be stopped. The reduction of the cupric salt and the precipitation of the cuprous salts will spread throughout the fluid from above downward. The urine should always be examined while fresh. If much albumin is present this should be removed.

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[59] Jour. of Biol. Chem., 1914, xvii, p. 519.

The reactions in the reduction of copper are as follows: If instead of urine pure water is used and the solutions of KOH and then of $CuSO_4$ be added, the first drop of the latter will precipitate $Cu(OH)_2[CuSO_4+2NaOH=Na_2SO_4+Cu(OH)_2]$. The blue flakes of $Cu(OH)_2$ will blacken when heated since they will be changed to $Cu(OH)_2 2CuO$. At this point it should be emphasized that only a salt in solution can be reduced. If at the onset a little glycerin or some tartrate solution had been added to the water all of the $Cu(OH)_2$ formed would remain in solution and so would not blacken when heated. If, instead of glycerin or the tartrate solution, glucose had been added to the water, a similar blue solution of the $Cu(OH)_2$ would be obtained (due to $C_6H_{12}O_6 5Cu(OH)_2$) but on warming this would be reduced and a yellow or red precipitate of cuprous salts would fall.

In the normal urine are certain bodies which, like glycerin and the tartrates, will hold $Cu(OH)_2$ in solution. Among these are the ammonia bodies, both those preformed and those resulting from boiling an alkaline urine, glucose and albumin if present. These, however, are not present in sufficient quantity to hold more than a drop or two of $Cu(OH)_2$ in solution and this trace when reduced will merely give a slight greenish color to the urine. If, however, to the normal urine or to the reagents used, glycerin, the tartrates, or an excess of ammonia be added, more or all of the $Cu(OH)_2$ formed will be held in solution and this will assume an azure blue color which varies in depth with the amount of $CuSO_4$ added.

But the normal urine contains also reducing bodies other than glucose which will reduce the copper on warming. Among these are uric acid, the glycuronic acid compounds, pyrocatechin, bile pigments, creatinin and a trace of glucose, always present. The sum of all these reducing bodies would not make up a solution of over 0.5% if expressed in terms of glucose. These bodies, if present in normal amount, will reduce enough of the copper to give a dirty yellowish, not a clear yellow color to the solution, but not enough to give a precipitate. Sometimes there is in the urine an increased amount of these substances (other than glucose) to give a definite precipitate of cuprous salts; that is, a positive test may result. But uric acid does not reduce copper at a temperature of from 60° to 70° C. as does glucose and creatinin reduces much copper only after long boiling, although it will a little at 60° C. The presence of these and other bodies (uric acid, creatinin, ammonia, albumin) is, however, very important since certain of them not only reduce copper but they all aid to hold in solution the small amount of the suboxides of copper which is always formed. Their ability in the normal urine to hold in solution these reduced suboxides is much greater than their ability to reduce copper and hence glucose may be added to normal urine even to a 0.5% solution before any cuprous oxide will precipitate.

In the urine of a case of glycosuria there is a great increase of the glucose and, because of the polyuria, a decrease in the concentration of those bodies which prevent the precipitation of the cuprous salts.

In performing Trommer's test it is important that the copper should not be added in excess since the black oxide formed by heat will mask the precipitate of the cuprous salts. In the case of normal urine from 3 to 5 drops of the $CuSO_4$ solution are sufficient to give a blue precipitate. In case sugar is present, however, one must continue to add the $CuSO_4$ solution until the first flakes of $Cu(OH)_2$ remain undissolved. For the test to be positive a yellow ($Cu(OH)_2$) or red (Cu_2O) precipitate must fall. If much sugar is present, metallic copper may be deposited on the glass (to clean such test-tubes strong nitric acid is recommended). In case there is less than 0.2% of sugar present no cuprous salt will precipitate, and yet the test may be very suggestive, because of the clear brilliant color of the yellow solution. Again, the precipitation of cuprous salts should occur at a temperature under the boiling point or promptly when the urine is just brought to that point to exclude the reduction by those bodies (creatinin, uric acid, etc.) normally present.

For a successful test the proportions of the reagents should be accurately estimated. Since 1 part of sugar can reduce about 5 parts of $Cu(OH)_2$, as nearly this amount of copper as possible should be present. Since glucose alone cannot dissolve as much of the $Cu(OH)_2$ as it could reduce were this in solution so glycerin, ammonia or the tartrates are added to Fehling's, Purdy's, etc., reagents, to hold in solution as much $Cu(OH)_2$ as possible in order that it may be at the disposal of the glucose. The optimum relation of reagents is 1 part of glucose to 5 (3 to 7) of $Cu(OH)_2$ and to 11 of NaOH. The excess of the last reagent is necessary since directly upon this does the temperature of reduction depend. If much too little NaOH be present, hours of boiling may be required to reduce the copper; if but 2 parts of NaOH to 1 of glucose are present a few minutes' boiling is enough, while if there is an excess of NaOH it is not even necessary to raise the solution to the boiling point in order to get a reduction.

Again, the best chance of a precipitation of the cuprous salts obtains when there is in the urine a minimal amount of those bodies which would hold the reduced copper salt in solution. For this reason it is advised by many, as a matter of routine, to dilute the urine about 1 : 5 with water, since this dilution will reduce the influence of these bodies in much greater proportion than it will the reducing power of glucose.

If to a urine rich in glucose considerable strong NaOH or KOH be added and only a little copper heat will produce a yellow, yellowish-brown or a dark-brown solution depending on the relative amount of sugar and alkali present (Moore's test). This dark color is due to the reaction of the alkali with that glucose in excess of the copper.

The result of Trommer's test in a normal urine may be a clear yellowish solution or a grayish-green shimmer due to a slight precipitate of the copper compounds of the xanthin bases and uric acid. The copper precipitated by sugar is crystalline, while that by the xanthin bases is amorphous. To be positive for glucose the precipitation should take place while the urine is still hot and not after it has cooled down. If but a trace of sugar is present the precipitate may fall after long boiling or after cooling, but such a reaction is not positive. A brilliant yellow color of the clear urine after boiling may suggest the presence of sugar but does not prove it. In such a case if the test is repeated after the urine is diluted a characteristic precipitate may fall. Since a normal urine reduces some copper, and would reduce more could it hold more of the $Cu(OH)_2$ in solution, a normal urine which has become ammoniacal may give a positive test for sugar since the ammonia will dissolve the cuprous salt. In a normal urine it is possible sometimes to get a positive test by adding an excess of NaOH and too much copper sulphate.

The phosphate precipitate always present may be stained slightly yellow by the $Cu_2(OH)_2$ formed even in normal urine. This often deceives.

In all copper tests a considerable amount of albumin would not hinder the reduction by glucose, but would the precipitation of the cuprous salt and hence should be removed unless but a trace is present, in which case it may be disregarded. Among the reducing substances present in the urine which may give a positive test for glucose are allantoin, mucin, pyrocatechin, hydrochinon, urobilin and perhaps also indican.

The test for glucose may be obtained when the glycuronic acid compounds are present in increased amounts, which is true following the use of chloral hydrate, chloroform, morphine, camphor, phenol, resorcin, thymol, and menthol. A positive reduction is obtained sometimes after the use of salicylic acid, benzoic acid, chrysophanic acid, oxalic acid, salol, thallin, santonin, copaiba, rhubarb, sulphonal, chloroform, acetphenetidin, glycerin and after poisoning with KOH, H_2SO_4, and arsenic. In alkaptonuria the test is positive. Saccharin hinders the reduction.

This is not as delicate a test as is Fehling's or Benedict's, and yet we have been interested to observe that those who have had the greatest experience in sugar work use Trommer's as a routine test since it gives much more information about the urine than the mere presence or absence of glucose. From it one may get a hint of the presence of other reducing bodies also.

Fehling's Test Solution.—Fehling's solution contains Rochelle salts which aids to hold a maximum amount of the cupric hydroxide in solution and so allows a maximum formation of the cuprous salts. Fehling's solution is made from 2 fluids, each quite permanent, but which must be kept separate, since an old mixed fluid may reduce on boiling.

Solution A	Solution B
Copper sulphate, 34.65 gm.	Rochelle salts, 173 gm.
Distilled water, q.s. ad 500 c.c.	Sodium hydrate, 125 gm.
	Distilled water, q.s. ad 500 c.c.

Equal volumes of these 2 fluids are mixed and brought to a boil. As soon as one is certain that the fluid will not reduce itself the urine is added to the boiling reagent slowly until precipitation occurs or the amount of urine added equals ½ that of the mixed reagent used (never more). If then there is no precipitate the test is negative for glucose. The mixture should be brought again to the boiling point after each addition of urine but prolonged boiling should be avoided. A precipitate which forms after the urine has been allowed to stand does not indicate sugar. By adding the urine slowly one can estimate pretty accurately the amount of sugar present. This test is positive for 0.08% of glucose. Although more delicate, it should be remembered that this test has all the faults of Trommer's. A normal urine will always reduce a little copper, but not if the urine is first diluted so that its specific gravity is about 1.005 (Zeehuisen).

Benedict's Test.—The following directions for making Benedict's reagent should be carefully followed.

	Gms. or c.c.
Copper sulphate (pure crystallized)	17.3
Sodium or potassium citrate	173.0
Sodium carbonate (crystallized) (one-half the weight of the anhydrous salt may be used)	200.0
Distilled water to make	1000.0

The citrate and carbonate are dissolved together (with the aid of heat) in about 700 c.c. of water. The mixture is then poured (through a filter if necessary) into a large beaker or casserole. The copper sulphate (which should be dissolved separately in about 100 c.c. of water) is then poured slowly into the first solution, with constant stirring. The mixture is then cooled and diluted to 1 liter. This solution keeps indefinitely.

Five cubic centimeters, a trifle over 1 teaspoonful, of the Benedict solution are placed in a test-tube and 8 to 10 drops (not more) of the urine to be examined are added. By using always these exact quantities very delicate results are obtained. The mixture is then heated to vigorous boiling, kept at this temperature for 3 minutes, and allowed to cool spontaneously. In the presence of glucose the entire body of the solution will be filled with precipitate, which may be greenish, yellow or red in tinge, according to whether the amount of sugar is slight or considerable. If the quantity of glucose be low (under 0.3%) the precipitate will form only on cooling. If no sugar is present the solution either remains perfectly clear, or shows a faint turbidity that is blue in color and consists of precipitated urates. Benedict states that the test if performed as above will detect glucose in as low concentration as 0.01 to 0.02% provided the urine is of low dilution.

Almén-Nylander's Test.—The Almén-Nylander solution is made up by dissolving 4 gms. of Rochelle salt in 100 c.c. of 10% NaOH (sp. gr. 1.015) warming, and saturating this solution with bismuth subnitrate (about 2 gms. are necessary). The solution is then cooled and filtered and kept in a dark bottle. It is permanent for years.

To the urine in a test-tube is added ⅒ its volume of this reagent. The fluid is then boiled preferably in a water bath for 5 minutes. If sugar is present the urine will turn black and a black precipitate of metallic bismuth will be deposited. If the urine contains over 0.2% of glucose the yellow color of the Moore test is first seen. Should the specimen become black only after it has been cooled the test is not necessarily positive. If but a trace of glucose is present, the white sediment of phosphates may be only slightly gray, especially on its upper surface. The boiling should be continued for fully 5 minutes, since only too often will the urine suddenly darken after one is tempted to pronounce it negative. If but a trace of sugar be present, the amount of urine and of reagent used be accurately measured. If this test is negative we may be sure that no sugar is present. If it is faintly positive, the result should be confirmed, since bismuth is reduced also by the quantity of paired glycuronic acid compounds sometimes present. This test is very delicate. It will indicate 0.05% (others say 0.025%) of glucose. Some claim that it is positive in 14% of normal urines especially if these are concentrated. Uroerythrin and hematoporphyrin both may give a similar test. Any modification in the reaction of the mixed reagent and urine injures the delicacy of the test, hence it should be applied carefully if the urine is ammoniacal since the NaOH of the reagent will replace the ammonia, which will be lost by volatilization leaving the solution not alkaline enough. Rhubarb and senna will reduce the bismuth salt but the fluid before heating will take a brownish-red color. This test is positive after salol, benzol, sulphonal, trional, antipyrin, kairin, much quinine, eucalyptus tincture and oil of turpentine. It is also positive after a person has eaten asparagus, a fruitful source of error. All albumin should be removed unless but a mere trace is present, since the Bi_2S_3, usually reddish in color, will, if precipitated in considerable amount, look black. This test is very valuable as a control of the copper tests, since this bismuth reagent is not reduced by uric acid, creatinin, pyrocatechin, hydrochinon, nor the alkapton bodies, and these are the greatest sources of error if copper solutions are used.

Fermentation.—The fermentation test is one of the best for the detection of glucose. If a busy clinician used this only fewer mistakes would be made. Only those sugars which have 3, or a multiple of 3, carbon atoms (and not all of these) will ferment with gas production. A piece of fresh, active yeast about the size of a pea is mixed with the urine in a beaker by rubbing it against the side of this vessel with a glass rod until the lump is well broken up. The urine should not be shaken during this process since enough air will be taken up later to give a bubble suspiciously large. The urine is then transferred to a fermentation tube and let stand for a few hours at a temperature of from 15° to 34° C. Two control tests should always be made: the 1 with normal urine to which the yeast and a little glucose have been added to prove the activity of the yeast; another fermentation tube is filled with normal urine plus a little of the same yeast to rule out the possibility that the yeast will undergo self-fermentation. All fermentation stops at 45° C. and over. If sugar is present the rapidity of gas production will depend to a certain extent on the amount of yeast added. The amount of gas formed from a given amount of sugar will, however, depend in part on the age of the yeast used since less will be formed by an old yeast. The maximum production of CO_2 (this would mean that 46.5% of the sugar was thus split) is obtained when for each gram of sugar is added not more than ½ a gram of fresh yeast. If too much yeast is used self-fermentation may result. This test if applied to a previously boiled urine will indicate from 0.1 to 0.05% of glucose.

Some consider it necessary to prove that the gas which collects in the fermentation tube is CO_2. This is easily done by dissolving it in NaOH. Some consider it necessary to prove that alcohol also is formed in this fermentation. This is easily done by distilling the fermented urine, adding to the distillate a little NaOH and some Lugol's solution, then warming and allowing the fluid to stand for several hours. Crystals of iodoform will appear if alcohol or acetone was present in the distillate. Or, to the distillate may be added a little very dilute solution of potassium bichromate and a little sulphuric acid. This fluid when heated will turn green and give off the odor of aldehyde.

Some bacteria will produce gas if the urine is allowed to stand too long. For that reason the acitivity of the yeast should be judged at the end of not over 6 hours. Or, bacterial growth may be inhibited by the addition of enough NaF to make a 1% solution, or by tartaric acid. Many recommend that the urine to be examined first be boiled for about 10 minutes to sterilize it and also to free it from air.

If but a trace of glucose is present in the urine no bubble of CO_2 may appear in the fermentation tube since urine can hold some of this gas in solution. For this reason a urine may be negative for glucose by the fermentation test and yet positive to Nylander's test, in which case the latter test should be repeated using the fermented urine which then will be negative.

Phenylhydrazin.—The phenylhydrazin test is theoretically the court of last appeal in the recognition of those carbohydrates which form with this reagent osazones of definite crystalline shapes and definite melting points. Albumin must be removed from the urine to be tested for it may hinder crystallization. Of the many methods of applying this test clinically that of Cipollina[60] is the best. In a common test-tube are mixed 5 drops of pure phenylhydrazin (the base), 0.5 c.c. of glacial acetic acid, and 4 c.c. of urine. This mixture, constantly shaken to prevent sputtering, is boiled over a low, free flame for about 1 minute, at the end of which time 4 or 5 drops of NaOH solution (sp. gr. 1.16) are added to reduce the acidity, although the fluid must still remain acid after the addition of the alkali. The liquid is then boiled again for a moment and cooled. The characteristic rosettes of crystals will form at once or at least within 20 minutes. The best results are obtained with urines of low specific gravity.

If much glucose is present the crystals of phenylglucosazon will form a yellow crystalline deposit in the tube. This may be filtered out, dissolved in hot 60% alcohol and recrystallized by adding water and boiling the alcohol away. The melting point of the crystals should then be determined. If pure, this lies between 204° and 205° C.; when impure, from 173° to 194° C. These crystals are yellow needles arranged in sheaves, which are difficultly soluble in water and in hot absolute alcohol, are easily soluble in 60% hot alcohol and which will crystallize out if water be added and the alcohol evaporated off. They are insoluble in ether, chloroform, etc., but are soluble in glacial acetic acid. Their solution is levorotatory.

A very simple method of determining the melting point of crystals is as follows (see Fig. 30): A small flask, *A*, is filled ¾ full with concentrated sulphuric acid. Through a perforated stopper is inserted a test-tube, *B*, also ½ full of the same acid. Into this dips a thermometer, *C*, to which is attached a tube, *D*, with a lumen about 1 mm. in diameter and sealed at the lower end which contains the dry crystals to be tested. This tube is attached by a rubber band to the thermometer. The flask is warmed slowly with a Bunsen burner and the point noted at which the crystals melt.

For other forms of apparatus the reaaer is referred to Menge's report.[61]

This test theoretically is very delicate and in simple solutions of pure glucose should show 0.003% of the sugar. Not all of the glucose is precipitated, the amount depending on the concentration of the glucose and the relative proportions of the reagents used. From a 5% pure glucose solution the maximum precipitation obtained by Fischer was from 85 to 90%. Much depends on the purity of the phenylhydrazin. In practical urine examinations this test is not delicate and seldom if ever indicates glucose in urines which will not reduce Fehling's solution.

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[60] Deut. med. Wchschr., 1901, Not. 21, p. 334.

[61] Bull. 70, Hygienic Lab., U. S. A., Oct., 1910.

The greatest danger of error when using this test is from pentose, but these crystals will melt at 159° to 160° C. The glycuronic acid compounds will form similar crystals but their melting point is lower, 114° to 115° C. All of the sugars which will reduce copper will give crystals, especially pentose and lactose.

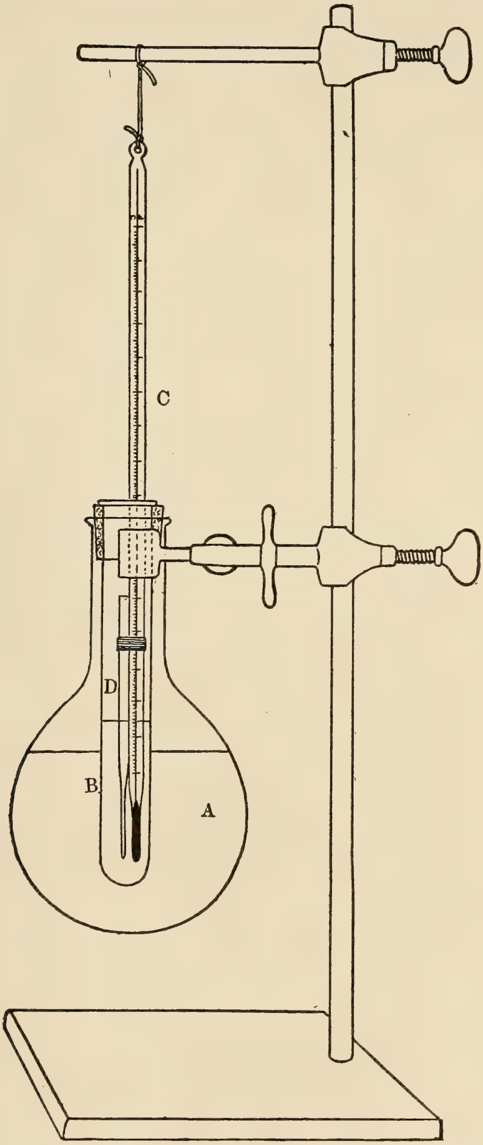

Fig. 30.—Melting point of crystals. *A*, flask, and *B*, test-tube of sulphuric acid; *C*, thermometer; *D*, fine-bore tube for crystals.

To form these crystals with lactose one must concentrate the urine and extract the lactose. Those sugars which differ only in the first 2 carbon atoms, the rest of the graphic formula the same, as, for instance, glucose, fructose, and mannose, all give exactly the same osozone. Crystals would be formed with acetone, hydrazin, oxalic acid, and uric acid, but in human urine not in sufficient amount to lead to error.

Zunz[62] considers the phenylhydrazin test one of great clinical value

Together with the crystals one finds also in the precipitate brown scales and oily droplets. These form even when a pure solution of glucose is used. This by-product is $C_{12}H_{12}N_2$, and can be removed by washing the sediment with chloroform or 95% alcohol and then recrystallizing the glucosazone from 60% alcohol. Sometimes even in the presence of glucose in the urine one gets a precipitate consisting only of brown scales or yellow amorphous granules or droplets. Such a result is to be considered negative, and yet glucose may be present.

In review it may be emphasized: that there is nothing characteristic in the shape of these crystals; their best solvent is 60% alcohol; they are best recrystallized by pouring the 60% alcoholic solution into water and evaporating off the alcohol; the hot glacial acetic acid solution, which is soon destroyed, may be tested with the polariscope; that in testing the melting point one cannot expect to find it exactly 204° to 205° C., so much depends on the purity of the crystals and on the speed with which the temperature is raised. Glucosazon differs from galacosazon in that its glacial acetic acid solution is levorotatory while that of the latter is optically inactive; otherwise they are very similar.

For the further separation of the carbohydrates of the urine we copy the table of Zunz.

The urine reduces Fehling's	Gives crystals with phenylhydrazin directly in urine.	Melting point of crystals about 200° C.	Fermentation positive.	Dextrorotatory.	Glucose.
				Levorotatory.	Levulose.
			Fermentation negative		Lactose.
		Melting point of crystals about 150° C.	Give orcin reaction.		Pentoses.
			Do not give orcin reaction.		Isomaltose.
	Gives crystals with phenylhydrazin only after the urine has been warmed with dilute sulphuric acid.				Paired glycuronic acid compounds.

The *polariscope* is a clinical instrument of great value in the recognition of sugars. It should be borne in mind that most normal urines are slightly levorotatory and that some urines which contain no dextrose are dextrorotatory (*e.g.*, Bornträger, in 2 morphia habitués) and so traces of sugar may escape detection or be wrongly reported as present. The ordinary instrument will detect 0.2% or more of glucose. The test is of greatest value if the urine be examined before and after fermentation

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[62] Jour. Méd. de Bruxelles, July 10, 1902.

Heat Test.—An easy test for glucose, sometimes valuable, more delicate than one would imagine, and always possible, is the following: one drop of urine is evaporated to dryness in a porcelain dish. It is then warmed gently. The residue becomes yellowish brown and at a temperature of 190° to 200° C. gives off the odor of caramels.

Moore's Test.—Moore's test was one of the first used for sugar. Its value now is not so much as a test but as a reaction which is often met with unexpectedly and should be correctly interpreted. To the urine is added ¼ volume of a strong solution of KOH or NaOH. On warming there results, first, a yellow, then an orange, and finally a dark brown color with an odor of caramels, more distinct if the urine is acidified. It may be necessary to boil for some time. This reaction develops slowly at room temperature. It is not a delicate test nor very accurate, since some normal urines will darken somewhat, also urines which are rich in mucus. The names glucinic acid and melasinic acid have been suggested for the substance coloring the urine.

Choice of Method.—If any of the above-described reduction tests is definitely positive, sugar is present. Of these, Nylander's is the one to be recommended first. If it is negative then no sugar is present. If positive, fermentation should next be tried. If this is positive the sugar probably is glucose, but levulose must be excluded. For the practitioner the fermentation test is perhaps the best test, since it leads to least confusion.

If one desires to clear the urine of glucose for further tests it must be remembered that glucose is not precipitated by sugar of lead but is almost completely by basic lead acetate.

Quantitative Determination of Glucose.—When sugar is known to be present in a specimen of urine and in good amount, an approximate quantitative estimate is possible by the use of Naunyn's table:

2 liters of urine of specific gravity 1028 to 1030 = 2 to 3%
3 liters of urine of specific gravity 1028 to 1032 = 3 to 5%
5 liters of urine of specific gravity 1030 to 1035 = 5 to 7%
6 to 10 liters of urine of specific gravity 1030 to 1042 = 6 to 10%

In estimating the amount of sugar from the specific gravity of the urine, and in using the following formula which employs the coefficient 230, it is assumed that changes in the specific gravity of urine are due to but 1 variable, *i.e.*, sugar. This is not strictly true since urea and the chlorides also are variables.

Suppose a diabetic patient voids 3 liters of urine in 24 hours and that this has a specific gravity 1.030. A normal person will void approximately 2 liters in 24 hours and the specific gravity would be 1.015. To determine the specific gravity of this normal person's urine were he to void 3 liters, we would calculate as follows:

$$\frac{2\times 1.015+1.000}{3}=1.010$$

$$1.030-1.010=.020$$

$$.020\times 230=4.6$$

That is, the urine of the above-mentioned diabetic patient would contain approximately 4.6% of glucose.

Supposing the patient voided in 24 hours 6 liters of urine with a specific gravity of 1.030.

$$\frac{2\times 1.015+4.000}{6}=1.005$$

$$1.030-1.005=0.025$$

$$0.025\times 230=5.8\%$$

Benedict's Quantitative Test.—Benedict's method is a great improvement over Fehling's and its several modifications. In Benedict's, as in Fehling's, method the glucose of the urine reduces a definite amount of copper in alkaline solution, thus decolorizing to a quantitative degree the blue copper solution. In Benedict's method, however, the copper is precipitated as cuprous sulphocyanate, a snow-white compound, and the end reaction is therefore much sharper than in Fehling's test. The solution for quantitative work, which keeps indefinitely, has the following composition:

Pure crystallized copper sulphate	18 gms.
Crystallized sodium carbonate (or 100 gms. of the anhydrous salt)	200 gms.
Sodium or potassium citrate	200 gms.
Potassium sulphocyanide	125 gms.
Five per cent. potassium ferrocyanide solution	5 c.c.
Distilled water to make a total volume of	1000 c.c.

With the aid of heat dissolve the carbonate, citrate, and sulphocyanide in enough water to make about 800 c.c. of the mixture and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water and pour the solution into the other liquid, with constant stirring. Add the ferrocyanide solution, cool and dilute to exactly 1 liter. Of the various constituents the copper salt only need be weighed with exactness. Twenty-five cubic centimeters of the reagent are reduced by 50 mg. (0.050 gm.) of glucose.

The procedure for the estimation is as follows: The urine, 10 c.c. of which should be diluted with water to 100 c.c. (unless the sugar content is believed to be low), is poured into a 50 c.c. buret up to the zero mark. Twenty-five cubic centimeters of the reagent are measured with a pipet into a porcelain evaporating dish (10 to 15 cm. in diameter), 10 to 20 gms. of crystallized sodium carbonate (or ½ the weight of the anhydrous salt) are added together with a small quantity of powdered pumice stone or talcum and the mixture heated to boiling over a free flame until the carbonate has entirely dissolved. The diluted urine is now run in from the buret, rather rapidly until a chalk-white precipitate forms and the blue color of the mixture begins to lessen perceptibly, then a few drops at a time until the disappearance of the last trace of blue color, which marks the end point. The solution must be kept vigorously boiling throughout the entire titration.

If the mixture becomes too concentrated during the process, water may be added from time to time to replace the volume lost by evaporation;

however, too much emphasis cannot be placed upon the fact that the solution should never before or during the process be diluted to more than the original 25 c.c. Moreover, it will be found that in titrating concentrated urines, or urines with small amounts of sugar, a muddy brown or greenish color appears and obscures the end point entirely. Should this be the case the addition of about 10 gms. of calcium carbonate does away with this difficulty.

The calculation of the percentage of sugar in the original sample of urine is very simple. The 25 c.c. of copper solution are reduced by exactly 0.050 gm. of glucose. Therefore the volume of diluted urine drawn out of the buret contains 50 mgms. of sugar.

When the urine is diluted 1 to 10, as in the usual titration of diabetic urines, the formula for calculating the percentage of sugar is the following: $\frac{0.050}{X} \times 1000 =$ percentage in the original sample, wherein X is the number of cubic centimeters of the diluted urine required to reduce 25 c.c. of the copper solution.

Chloroform should not be present in the urine during the titration. If it had been used as a preservative it may be removed by boiling a sample for a few minutes and then diluting to the original volume.

To determine the glucose in a specimen of urine containing very little sugar, about 1500 c.c. of the urine is precipitated with sugar of lead and the filtrate then precipitated with basic lead acetate and a little ammonia. This precipitate is suspended in alcohol and decomposed with H_2S. The filtrate is then cleared with animal charcoal if necessary and evaporated at low temperature to a small volume. The amount of glucose in this solution is then determined with the polariscope. A correction must be made for lactose or bile acid if present, both of which are dextrorotatory. To do this the alcohol after the specimen is polarized is evaporated off, the residue dissolved in water, yeast added and the glucose removed by fermentation. This fluid is then filtered, the precipitate washed with alcohol, filtered, the filtrate restored to the original volume, and it is again polarized. From the difference between these two readings the amount of glucose may be calculated.

Polariscope.—For this very important test and quantitative method the specimen must be very clear, so clear that even fine type may very easily be read through the tube of the polariscope full of the urine. All albumin must be removed since it is levorotatory. The urine is best cleared, if possible, with infusorial earth. An excess of this is added to the urine which then is well stirred and filtered. The filtrate should be poured back into the funnel until it runs clear. If, as sometimes happens, this method does not clear the urine perfectly, crystals of sugar of lead are to be added to the infusorial earth and the urine filtered through this mixture.

Sugar of lead in crystals is somewhat preferable to it in solution since the latter would change the volume of the urine. Yet some prefer to add to 90 c.c. of the urine 10 c.c. of a PbAc solution (25 gms. in 100 c.c.) and then filter. This clears the urine perhaps better. The proper correction for the change of volume is easily made. Basic lead acetate cannot be used. Infusorial earth is to be recommended since lead acetate in any excess will alter the physical properties of the urine and will certainly remove some of the glucose, although it would none from a pure glucose solution. Yet infusorial earth also may remove some of the sugar. Others recommend that the urine be cleared with a small amount of PbAc and a teaspoonful of Na_2SO_4 (added after the sugar of lead is dissolved).

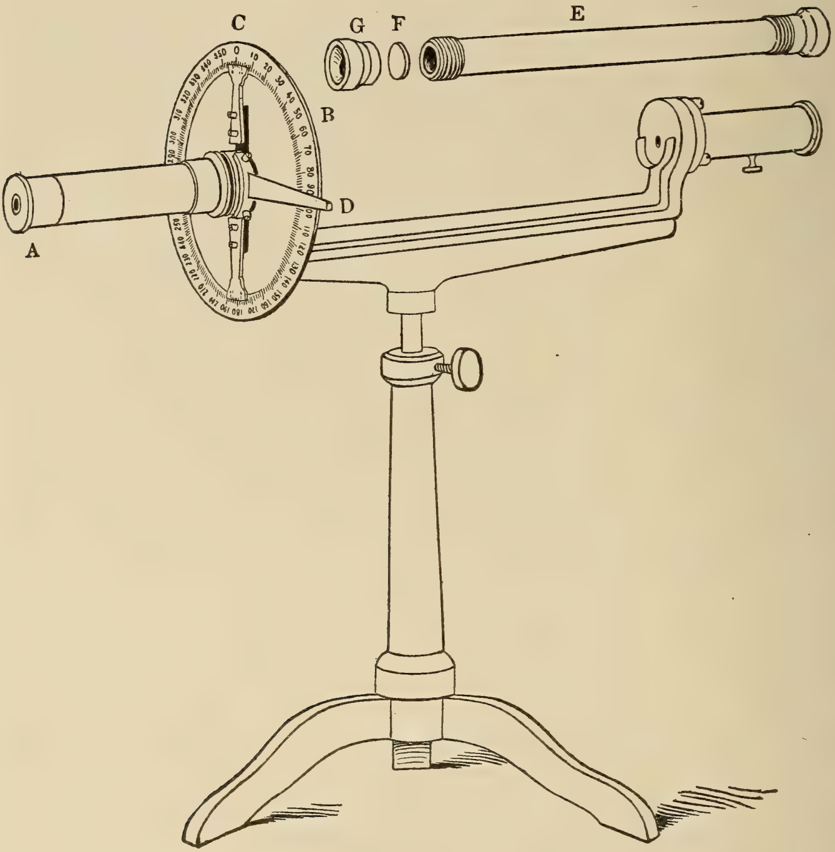

FIG. 31.—Half-shadow saccharometer. *A*, ocular used in focusing the field; *B*, graduated disk; *C*, vernier; *D*, lever for rotating analyzer; *E*, tube for urine; *F*, glass disk; and *G*, cap for end.

The tube of the polariscope is filled with the clear urine, care being taken that no air-bubbles be enclosed, and the angle of rotation measured by the scale on *B* (Fig. 31), using the vernier *C*, to read the fractions of a degree.

The tube, *E*, is first cleaned thoroughly and dried. The glass disks, *F*, are made perfectly clear. One glass is fastened in place and then the tube filled with the perfectly clear urine till the meniscus is convex. The second glass disk is then slid in position from the side, pushing off the excess of urine and leaving no air beneath. The metal cap, *G*, is then screwed down over this.

The student should understand thoroughly the instrument that he is using. There is a great variety of polariscopes on the market which differ slightly in their construction and more in their accuracy. It is seldom, of course, that a standard polariscope is used. The most are instruments modified for clinical purposes. The polariscope is an instrument which measures the angle of rotation of polarized lights caused by an optically active substance; the length of the standard tube is 10 cm. or a multiple of this and the readings are made in degrees. The specific rotation of glucose is $(\alpha)_D = 52.74°$. If a polariscope is used, therefore, to examine urine the angle of rotation must be divided by 0.527 to give the percentage of sugar in the urine. The instruments in clinical use are usually "half-shadow instruments" whose tubes are of such length (188.6 mm. but better 189.4 mm.) that 1° of rotation will indicate 1% of glucose. Each instrument usually has a second tube, ½ as long (94.3 mm., better 94.7 mm.) for highly colored urines. Another instrument which has become very popular and which is more convenient to use is the saccharometer in which the rotation by the sugar is balanced by a compensating quartz wedge on which is marked an empirical scale. The great advantage of this instrument is that an ordinary white light, as the Welsbach burner, can be used while in the other instrument a sodium flame alone is admissible. One disadvantage of this instrument for teaching purposes is that the principles in optics involved are not as evident to the student.

In using the half-field polariscope the field must be first focused at *A* and the zero point determined. This changes somewhat with the temperature, particularly in a carelessly used instrument. The tube is then inserted, the field again focused sharply, and the rotation determined. The accuracy with which this can be done will depend upon the clearness of the field, which in turn will depend on the fluid, the focus, the sensitiveness of the instrument, and the brightness of the light. There are two methods in common use of finding the end-point at which the fields are of equal illumination. According to one the analyzer is rotated until a black band seems to cross the division of the fields. This shadow, purely subjective, always appears a little too soon, therefore several readings from both directions should be made and averaged. According to the second method the analyzer is slowly turned, always in the same direction, and using the eye but for a few seconds at a time, until the end-point seems to be just reached. This point also will be attained a little too soon, hence several readings should be made, turning the analyzer from both directions, and an average taken. In all cases it should be remembered that the eye should be used for not over 15 seconds at a time to avoid fatigue of the retina. The depth of the illumination of the whole field should be judged and not of the contiguous portions of the 2 shadows (see Fig. 32).

Many of these half-shadow instruments are so sensitive that they indicate a difference of even 0.02°, but since the error inherent in the urine itself is even 0.2% it is evident that very minute readings have only the appearance of accuracy.

The surface of the ends of the tube must be planned accurately to a right angle to its long axis, otherwise the tube cannot be used. This error can easily be detected by putting the empty tube in the instrument, focusing carefully, and then revolving the tube around its long axis, which will produce the same effect as though the analyzer were rotated. Leather washers are necessary between the metal caps and the glass disks to prevent tension in the glass from the pressure of the cap, since glass subjected to too high tension becomes doubly refractive. For this reason, before any readings are made, the tube properly inserted in the instrument should be rotated around its long axis and the effect watched through the analyzer. If this rotation causes the 2 fields to change in relative intensity 1 of the above mentioned sources of error is present. Differences in the intensity of illumination of the whole field may indicate either that the solution is not homogeneous or that the tube is dirty.

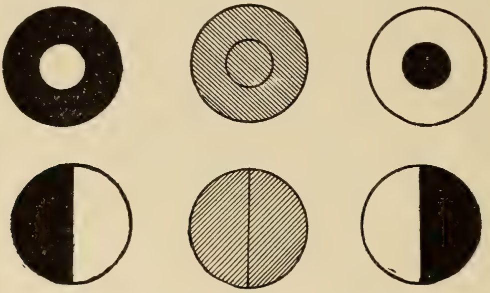

FIG. 32.—The fields as seen in the two most common types of clinical saccharometers. The central figures, gray fields with halves of equal illumination, are the zero points. The others are the fields with too little and too much rotation.

Normal urine is slightly levorotatory (0.005° to 0.18°). A trace of sugar may therefore be present and cause no dextrorotation. In some cases the urine is dextrorotatory when glucose is not present. Such was true of 2 patients with the morphia habit (Börntrager).

On the whole this is the quickest practical qualitative method of sugar analysis. Albumin must be removed from the urine since it is levorotatory. Should the worker have occasion to make up a glucose solution as a control he must remember to use in the polariscope a solution which has stood for at least 1 day, since glucose when first dissolved shows some birotation.

Fermentation.—According to one method (Robert's method) the amount of sugar in the urine is estimated from the difference in the specific gravity of the urine before and after fermentation. The urine should first be acidified, if necessary, with tartaric acid. The specific gravity is then carefully determined, using a very accurate aërometer and making the proper correction for the temperature of the fluid. A piece of washed yeast the size of a hazelnut is then added and the urine allowed to ferment at from 15° to 35° C. (a temperature of 34° C. is the best) until it gives no qualitative test for sugar. This usually takes from 24 to 48 hours. The sediment is then brought into suspension and the specific gravity of the urine again tested. The difference between these specific gravities multiplied by 234 will give the percentage of sugar. For this determination it is best to use the pycnometer method to determine specific gravity, since the aërometrical method, which at the best is poor, is hardly delicate enough for this work. If accurately applied the results are correct to about 0.1%. Albumin need not be removed. This method can be used if 0.5% or more of sugar is present. This is the best method for the practitioner who has not a polariscope nor the time to prepare for and to carry out the titration method. Yet unless one is careful in the details, an error of even 5% may be made.

Fermentation: Gas-Volumetric Method.—The Einhorn method of estimating the amount of sugar in a specimen of urine by measuring the amount of carbon dioxide produced by fermenting it with yeast is much more accurate than is generally believed, provided one uses a fresh yeast and pays due attention to temperature, etc. Lohnstein's [63] instrument is said to be accurate and to give the final result in 6 hours.

Levulose is a sugar which is met with widely in the vegetable kingdom, particularly among the fruits. It is often present in the urine of diabetics, but practically always in company with glucose. An alimentary levulosuria can usually be produced by injecting or feeding levulose to those cases with liver trouble which seem to disturb the ability of this organ to transform levulose to glycogen (see page 182). In addition to these 2 forms of levulosuria there are on record a few, 6 or 7 only, cases of pure levulosuria (Naunyn) with even from 1 to 2% (as a rule, less than 1%) of this sugar in the urine. Rosin and Laband reported [64] an interesting case of pure levulosuria with about 0.6% of levulose in the urine. This patient showed a levulosemia of 0.5% even when the urine was negative. The levulosuria of this patient was uninfluenced by the ingestion of even 100 gms. of levulose or glucose. In a case reported by Strouse and Friedman [65] the levulose of the urine seemed to be entirely exogenous and derived from ingested levulose and from higher carbohydrates, which contained the levulose molecule. The levulosuria of this patient was not affected by the glucose ingested nor was his tolerance for glucose decreased. Levulose can be used easily by some diabetics, but by no means all. As a rule if levulose is ingested in large amounts almost all of it is excreted as glucose.

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[63] Münch. med. Wochenschr., 1899, No. 50.

[64] Zeitschr. f. klin. Med., 1902, vol. xlvii, p. 182.

[65] Arch. Int. Med., January 15, 1912, vol. ix, p. 99.

The diagnosis of many of the reported cases of levulosuria is doubtful, since levorotatory bodies often present in the urine were not excluded. Levulosuria is to be suspected when the percentage of sugar as determined by polarization is definitely less than that which titration would indicate and when the levorotatory body is found to be fermentable with gas production.

The most important levorotatory sugars are laiose and fructose. Fructose gives reactions very similar to those of glucose; it reduces copper somewhat less readily (10%), it ferments with gas production and has an angle of levorotation of uncertain amount. Its characteristic test is that of Seliwanoff, although Rosin, also Fr. Müller [66] state that glucosamin also will give this test. To a warmed solution of resorcin in moderately dilute hydrochloric acid (1 volume of HCl to 2 volumes of H_2O) is added a little of the sugar in question. If it be levulose the fluid at once takes on a beautiful red color, due to a substance which precipitates on cooling and which is soluble in alcohol. Levulose, if warmed with a concentrated alcoholic solution of resorcin, gives a brick-red color. It gives the same osazone as glucose. It is a more fragile body than is glucose.

The other levorotatory bodies of the urine which must be excluded are albumin, glycuronic acid compounds, β-oxybutyric acid, and cystin. If the levorotation of a urine disappears on fermentation, the chances are that levulose was present. This should be confirmed, however, by isolating the sugar and testing it in pure solution.[67]

The alimentary levulosuria test for functional disturbance of the liver has attracted considerable attention. Strauss [68] found that the ingestion of 100 gms. of levulose was followed by a levulosuria in 90% (26 of 28 cases) of the cases with hepatic trouble which he studied, and in but 11% (6 to 58) of the normal men. Ferrannini and Bruining considered the test valuable, but Landsberg [69] could obtain the test in but 9 of 21 cases of liver trouble (not severe ones), and in 4 of 7 normal persons. He therefore doubts that it has any value.

Lactose is found in the urine of the great majority of women during lactation. Ney found it in 115 of 148 cases, others in all. The output reaches its maximum on the second to the fourth day after delivery. The amount eliminated usually is small, but it may reach even 2 to 3%. In these cases the sugar is absorbed from the lacteal glands. Lactose appears in the urine also of patients who for a long time have been on a milk diet. It will as a rule appear in the urine also after the ingestion of 100 gms. of this sugar. Voit found that if lactose be fed to diabetics they excrete more glucose, while in the case of lactating women this is not true.

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[66] Deutsches Arch. f. klin. Med., 1904, p. 1630.
[67] See Peligot method, Compt. rend., vol. xc, p. 153.
[68] Deutsch. med. Wochenschr., 1901, Nos. 44 and 45.
[69] Ibid., August 6, 1903.

Lactose is dextrorotatory (52.5°). Lactosazon crystallizes in small yellow prisms arranged in spheres whose melting point is 200° C. (This test cannot be applied to urine directly. The urine must be concentrated and the lactose extracted.) Its reduction tests are similar to those of glucose, but copper is reduced somewhat less actively and ammoniacal silver nitrate is reduced without the aid of heat. Nylander's test is positive. If a solution of lactose is boiled for several hours with dilute mineral acid, the lactose will be inverted to galactose and glucose. Lactose is not fermented by ordinary yeast. It can be fermented but without the production of CO_2. The presence of lactose in the urine is to be suspected when the copper and bismuth tests are positive yet somewhat slow, and the fermentation and phenylhydrazin tests are negative. Urine to be tested for lactose should first be sterilized, otherwise bacteria will split lactose giving rise to a fermentable sugar. If a urine does not ferment with gas production and yet does reduce copper, lactose or pentose should be suspected.

Rubner's test for lactose deserves special mention. If urine containing lactose is boiled from 3 to 4 minutes with an excess of sugar of lead it becomes yellow or brown in color. While still hot, ammonia is added until the precipitate which forms no longer dissolves. From the intense brick-red fluid which results, a copper-red precipitate will separate, leaving the supernatant fluid colorless. If the specific gravity of the urine to be tested is over 1.020 it is wise to dilute it one-half. Glucose under these conditions would give a red solution with a yellow precipitate; maltose, a yellow solution, and levulose, no change of color at all.

Pentoses.—The pentoses are a very important group of sugars with a chain of 5 carbon atoms. These are rather complex carbohydrates from which pentose may be split by hydrolysis are widely distributed in the vegetable kingdom. In the metabolism of the herbivora the pentoses play almost the same role as the hexoses in man, in that they are glycogen-builders. Pentose also is important since it is the carbohydrate nucleus in the nucleo-proteid molecule of certain organs, the pancreas, thyroid, thymus, brain, spleen and liver.

In the following paragraphs we shall quote the literature of this subject, warning the reader, however, that although the observations made by many may be accurate yet the conclusions of those who would interpret these observations as proving that the sugars in question were pentoses may not have been sufficiently confirmed.

PENTOSURIA is said to occur under 3 conditions. First, an alimentary pentosuria will in normal persons follow the ingestion of considerable amounts of fresh fruit juice, or of beer. In these cases it is always an optically active pentose which is eliminated. Of all sugars the pentoses have the lowest assimilation limit. A dose of even 50 mgms. of pure xylose, *e.g.*, will produce a pentosuria. Second, some diabetic patients have pentosuria as well as glycosuria. It is only in very severe cases that the inability to burn sugars extends also to pentose (Kulz and Vogel found pentoses in the urines of 64 of 80 cases). V. Jaksch found that diabetics excrete from 48.98 to 82.02% of the arabinoses, but a trace of the xylose and from 3 to 13% of rhamnose of the food, while non-diabetic patients excrete from 1 to 46.65% of arabinose, from 54.8 to 18.7% of xylose and from 63 to 55% of the rhamnose of the food.

Third, there are on record a few cases of idiopathic pentosuria, a condition quite different from diabetes. This was first discovered by Salkowski and Jastrowitz in 2 cases of suspected glycosuria. In 1902 only 5 or possibly 6 [70] such cases had been reported. Bendix later collected 12 cases and added 1. It is interesting that several of these were chronic morphia habitués. In 1 of these the pentosuria continued after the habit was cured, but did not in another.

Janeway [71] collected 22 cases of pentosuria and added 2 who interestingly enough were brothers.

Pentosuria would seem to be a chronic symptomless condition discovered usually by accident. The amount of pentose on the urine is not large. In Salkowski's case the amount eliminated would correspond to from 0.07 to 0.15% and in Bendix's case, to from 0.4 to 0.6% of xylose. Bial [72] found that in such cases the assimilation limit for both glucose and pentose is normal. The only explanation for the condition that he can offer is that there is an excess of pentose formed.

Urines containing pentose will reduce copper, but not readily. The routine tests would suggest that a trace only of glucose or lactose is present. The reduction develops slowly after cooling, and suddenly throughout all the urine. Such urines do not ferment, are slightly dextrorotatory, and give with Nylander's solution a gray precipitate.

Xylose, the most important clinically of the pentoses, is dextrorotatory, does not ferment with gas production, forms osazones with a melting point of 159° to 160° C., reduces copper and Nylander's solutions, gives an orange precipitate with Ruberner's test, and positive furfurol reaction.

The *arabinoses* reduce copper and Nylander's solutions somewhat better than does xylose, and form osazones, the melting point of which varies from 157° to 158° C. One of the arabinoses is dextrorotatory but it is the inactive arabinose which is of particular interest, since this is the sugar found in the urine of the idiopathic cases.

The Phloroglucin Reaction.—To a few cubic centimeters of the urine are added an equal amount of HCl (sp. gr. 1.19) and then from 25 to 30 mgms. of phloroglucin. The solution is then warmed until it becomes red in color.

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[70] Brat's case, Münch. med. Wochenschr., 1903.
[71] Am. Jour. Med. Sc., Sept., 1906.
[72] Verh. d. xix. Kongr. f. inn. Med.

This solution if examined at once spectroscopically will show a band in the green if the pentoses are present.

Salkowski recommends the following modification of this test: Five to six cubic centimeters of fuming HCl are warmed and saturated with phloroglucin, leaving some undissolved. This solution is then equally divided in 2 tubes. To the 1 is added 0.5 c.c. of the suspected urine, and to the other 0.5 c.c. of normal urine. Both test-tubes are then placed in a beaker of boiling water. The tube containing pentose will soon be red in color, the change in color beginning above and extending downward. The tubes should be removed from the water-bath as soon as the red color begins to appear, and the red fluid examined spectroscopically.

To exclude glycuronic acid, the pentosazone must be obtained (Salkowski). To from 200 to 500 c.c. of urine in a beaker are added 2.5 gms. per 100 c.c. of phenylhydrazin dissolved in an excess of acetic acid (or 3.5 gms. HCl phenylhydrazin with 1.5 parts of NaAc). This fluid is then warmed to the boiling point, the beaker allowed to stand in boiling water from 1 to 1¼ hours and then cooled. If pentose is present in any considerable amount an abundant sediment of crystals will separate out. As soon as the crystallization is complete the precipitate should be recrystallized from a hot, very dilute alcoholic solution, and this repeated until the melting point is constant.

The *orcin-HCl test* is to be preferred since it is somewhat more specific. To the urine, decolorized with animal charcoal, is added an equal volume of concentrated HCl, and then a small amount of orcin. This solution is then heated. If pentose or glycuronic acid (liberated from its paired compound by the acid and heat) is present, the color of the fluid turns to a reddish-blue (although the red may be very transitory and sometimes not appear at all) and finally become green. The solution is then cooled until it is only warm, and is then shaken out with amyl alcohol. A green fluid with a characteristic absorption spectrum is obtained.

Bial's modification of the Salkowski-Blumental test[73] is as follows: A reagent (HCl, 30%, 500 c.c.; orcin, 1 gm.; 10% Fe_2Cl_6, 25 drops) is used. Four or five cubic centimeters of this reagent are heated to boiling, then removed from the flame and the urine under examination added drop by drop, the total amount added not to exceed 1 c.c. If pentose is present a fine green color will soon appear. Bial claimed that this test excludes the glycuronic acid compounds, in fact is given by pentose alone.

If hexoses also are present in the urine they, together with the pentoses, should be precipitated as osazones and the separation then made. The attempt should not be made to remove them by fermentation since the pentoses also will disappear during the process possibly as the result of bacterial action.

Method of Kulz and Vogel.—From 1.6 to 3.2 liters of urine are used. To it are added 200 gms. of phenylhydrazin plus 100 gms. of glacial acetic acid for each 100 gms. of glucose in the urine. The urine thus treated is then heated on a water-bath for an hour and a half, cooled and filtered. The filtrate is again heated on the bath for 1½ hours and filtered. These combined precipitates are well washed with cold water and then digested in water at 60° C., which will dissolve the pentosazone. (Glucosazone can be dissolved only if the heat is raised to the boiling point.) For this digestion of the crystals 1 liter of water per 100 gms. of sugar is used, and the digestion continued 12 hours. This is repeated 15 times. The hot extracts are then filtered and allowed to cool, during which process the pentosazone will separate out. This precipitate is repurified, using less water, until the melting point of the crystals is constant.

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[73] Deutsch. med. Wochenschr., July 2, 1903.

The separation of the different pentoses is made with the polariscope. The alcoholic solution of zylosazone will show a strong constant levorotation, while arabinosazone immediately after formation is dextrorotatory and later becomes optically negative.

One reason why cases of so-called pentosuria would seem to be rare may be that they are overlooked, or, unfortunately for the patients, usually diagnosed as glycosuria. But it is also true that pentosuria may prove to be a term covering a heterogeneous group of cases the urines of which give reactions suggestive of pentose, but, as certainly is the case, in some it is not pentose.

Inosite.—Inosite is a carbohydrate-like body (really a hexahydroxylbenzol, $C_6H_6(OH)_6H_2O$) which is widely distributed in the vegetable kingdom. It is sometimes present in small amounts in the urine of patients with nephritis and diabetes and other conditions with polyuria. Naunyn mentions a case, probably of diabetes insipidus, who eliminated 18 to 20 gms. of inosite per day. Hoppe-Seyler believed that it may be found in all normal urines.

The albumin should first be removed, the urine evaporated to ¼ its volume and then precipitated with baryta water. The precipitate is washed, decomposed with H_2S and filtered. The filtrate is allowed to stand in order that the uric acid may precipitate and be removed by filtration. The filtrate is then concentrated to a syrup and treated while boiling hot with 2 to 3 volumes of alcohol. A precipitate rapidly forms. This is cooled and ether is added after which the crystals of inosite will slowly appear. These are then purified by decolorization and recrystallization.

Scherer's Test.—Inosite is evaporated to dryness with nitric acid on a platinum foil. To the residue are added a little ammonia and 1 drop of $CaCl_2$. It is then again evaporated to dryness and a fine rose-red residue obtained. This test is satisfactory only when the inosite is fairly pure.

Seidel's Test.—This is the same as the above except that strontium acetate is used instead of $CaCl_2$. A fine green colored solution with a violet precipitate develops. This test is positive even when but 0.3 mg. of inosite is present.

Gallois Test.—The inosite solution is evaporated almost to dryness and the residue moistened with a little mercuric nitrate. On drying the solution a yellow residue is obtained, which at high heat becomes of a fine red in color, which color disappears on cooling and reappears on warming.

Glycogen (Erythrodextrin).—This has been found in the urine of diabetics after the glucose has disappeared or diminished, as a dextrin-like substance which browns on the addition of iodine. Urine containing it reduces copper after long boiling. To detect glycogen the urine is evaporated to a syrup, and KOH and absolute alcohol added until a cloud due to the potassium salts appears. The fluid is then decanted, the precipitate is washed several times with absolute alcohol, dissolved in acetic acid, and reprecipitated with absolute alcohol. This precipitate is warmed with the alcohol and dried. A white tasteless powder is obtained, soluble in water, which reduces copper slowly and browns on the addition of iodine.

Animal Gum (Landwehr).—Animal gum is said to be present in normal urine. It seems to be of the nature of a pentose, is slightly dextrorotatory, and does not ferment with gas production. With the copper test one obtains a precipitate which on boiling does not blacken. Alfthan found it much increased in the urine in diabetes mellitus (1.2 to 36.9 gms. per day; normally from 0.1 to 0.2 gm.). It is probably not 1, but a group of bodies precipitable by alcohol.

Laiose is a sugar the nature of which is uncertain, found by Leo[74] in the urine of some, but not of all, diabetics. It is levorotatory, non-fermentable, with a salty taste and little reducing ability except after long boiling. It gives an oily compound with phenylhydrazin.

Maltose was present in small and varying amounts in the urine of two patients.

Isomaltose.—This sugar has been demonstrated in normal urine. Whether preformed or formed from glucose is uncertain. Certainly this transformation is very easy. The osazone precipitates in the form of very fine crystals which have a melting point of 150° to 153° C. It either does not ferment or if it does it is very slowly, it reduces copper and bismuth and is dextrorotatory. It may be demonstrated as a benzoate compound.

Melituria.—In the case of some malingerers it may be necessary to test the urine for cane sugar. In Brown's[75] case the urine had a high specific gravity, gave a positive but unsatisfactory Fehling's test, and but very few crystals with phenylhydrazin. It fermented very slowly. It was dextrorotatory. These tests would suggest that there may have been a trace of glucose in this urine, but it is more likely that some of the cane sugar had been inverted by the acid or by the bacteria of the urine. To demonstrate cane sugar the urine is concentrated, boiled with dilute HCl for from 20 to 40 minutes and neutralized with sodium bicarbonate, after which the typical tests of glucose and of levulose may be obtained.

Acetone.—There is some acetone in all normal urines, the maximum normal output being about 10 mg. in 24 hours. It is much increased in the following conditions:

(1) *Alimentary Disturbances.*—The acetone of the urine is increased whenever the carbohydrates of the diet are much reduced. It is always increased by a diet rich in proteid and hence also during hunger periods. It is increased also by a diet rich in fat, although it required the ingestion of about 150 gms. of fat to influence the output. That an acetonuria may be of gastro-intestinal origin and is often associated with indicanuria is suggested by Wilson.[76]

(2) *Febrile Acetonuria.*—An acetonuria may be met with in any case of fever, no matter how slight. It has no clinical importance.

(3) *Diabetes.*—In diabetes the output of acetone in the urine is the largest of all conditions. An intense acetonuria means an advanced, a long-standing case, and usually a severe case. While there are exceptions to this rule and while its presence need not necessarily mean an unfavorable prognosis, the curve of the acetone output is of some value in following an

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[74] Virchow's Arch., 1887, vol. cvii, p. 108.

[75] Johns Hopkins Hosp. Bull., May, 1900, p. 101.

[76] The J. of Lab. and Clin. Med., May, 1920, v. 515.

acidosis. A severe case may eliminate more than 5 gms. of acetone daily. The acetonuria may increase greatly following slight fever; it is decreased by the administration of alkalies; the output of cases on rigid diet may be reduced by adding carbohydrate to the food. Finally, it tends to increase as coma develops and toward death. Traces of acetone are eliminated also by the breath, 150 mgms. even being excreted in 1 hour through the lungs.

Folin [77] has upset a few of the generally accepted ideas concerning acetone. He finds that its amount in the urine of even severe cases of diabetes is very small indeed; that the most of the substance estimated as acetone is diacetic acid; that some of the tests of acetone, *e.g.*, Legal's, are really very delicate tests of diacetic acid; and that while the diabetics' breath and urine may contain acetone and may have a fruity odor, yet this odor is not due to acetone.

(4) *Patients with carcinoma* in which inanition has not yet begun. (5) *Cases of inanition and cachexia.* (6) *Psychoses* and lesions of the central nervous system, especially those associated with starvation. (7) "*Autointoxication.*" (8) *Digestive disturbances*, especially gastric ulcer. (9) *Chloroform narcosis*, in which case it is due to the increased proteid catabolism. (10) *Pregnancy with a dead fetus.* (11) *Certain poisons: e.g.*, phloridzin. (12) *Extirpation of the pancreas.*

There is some doubt that there is any preformed acetone in the body. All in the urine would seem to be derived from diacetic acid.

Acetone, CH_3COCH_3, is a thin, colorless fluid with a specific gravity of 0.814 (at 0° C.), a boiling point of 56.5° C. and a quite characteristic odor.

Tests.—As a general rule only the distillate of the urine should be tested for acetone. From 250 to 1000 c.c. of fresh urine are used and a little acid, preferably phosphoric, added to prevent foaming. V. Jaksch advises that it be distilled with steam, in which case no acid is necessary. A good cooler should be used if the acetone is to be determined quantitatively, although for qualitative work this is not necessary. Most of the acetone will pass over in the first 10 to 30 c.c. of the distillate. Since diacetic acid is easily split up to acetone the urine should first be made alkaline and carefully shaken out with alcohol-free ether if it is desired (as it seldom is) to exclude this body. This ether extract may then be shaken out with water and the latter tested for diacetic acid.

Legal's Test.—As a preliminary test, Legal's may be used and yet this is satisfactory only when large amounts of acetone are present. A negative result has little value.

To the urine or its distillate are added a few drops of fresh concentrated solution of sodium nitroprusside, and then KOH or NaOH until the reaction is very alkaline. A ruby-red color appears which changes rapidly to yellow. The test thus far is the same as that given by creatinin. Glacial acetic acid therefore is added in excess to the still red fluid. If acetone is present the red will change to a purple-red and later to a violet color. Creatinin would give a yellow color which would change to green and finally to blue. Paracresol gives a reddish-yellow solution; acetic acid a clear rose color. To exclude aldehyde Le Noble and Lee used NH_4OH in place of KOH.

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[77] Jour. of Biol. Chem., May, 1907; Jour. of A. M. A., May 2, 1908.

Gunning's test is very satisfactory and accurate. To the distillate is added tincture of iodine or Lugol's solution (KI, 1.8; I, 1.2; H_2O, q. s. ad 30), and then ammonia until a deep black precipitate forms. This later gradually disappears, leaving a yellow sediment of hexagonal or star-shaped iodoform crystals which may be recognized by their color, odor and shape (see Fig. 33). The sediment is seldom amorphous. In case but a trace of acetone was present it may be necessary to wait 24 hours before the sediment appear. If necessary the iodoform may be recrystallized from ether. Gunning's test is less delicate than Lieben's, but is given by no other body than acetone, of which it will show 0.01 mgm. per 1 c.c.

Denigè's test[78] is preferred by some: To about ½ inch of the distillate of the urine in a test-tube is added an equal amount of a solution of the subsulphate of mercury (mercuric oxide, 50; sulphuric acid, 200; water to 1000). This mixture is allowed to simmer about 5 minutes. When it cools there is deposited a white crystalline precipitate, which is distinctive in appearance, and is not soluble in dilute HCl.

A test now popular is as follows:[79] To 10 c.c. of urine in a test-tube one adds about 1 gm. of ammonium sulphate, 2 to 3 drops of a freshly prepared 5% solution of sodium nitroprusside, and 2 c.c. of concentrated ammonium hydroxide which may be stratified or poured on the mixture. The presence of acetone is indicated by the slow development of a permanganate color. The test is positive if there is 1 part of acetone in 20,000 parts of urine.

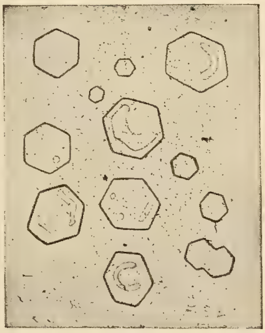

FIG. 33.—Iodoform crystals formed from the distillate of the urine of a case of diabetes.

QUANTITATIVE DETERMINATION OF ACETONE PLUS DIACETIC ACID.—The Huppert-Messinger Method. By this method one determines the sum of the acetone and of the diacetic acid which is transformed to acetone by the process of distillation.

The solutions necessary are:

1. Acetic acid, 50%.
2. *0.1N* iodine solution.
3. *0.1N* sodium thiosulphate solution.
4. A thin starch solution (1 gm. of starch dissolved in 500 c.c. of boiling water).

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[78] See Taylor, Jour. of A. M. A., March 17, 1906, vol. 46, p. 790.
[79] Wilson, Jour. of Clin. and Lab. Med., 1920, v. 515.

To make up these solutions 24.8 gms. of crystallized sodium thiosulphate ($Na_2S_2O_35H_2O$) are carefully weighed, dissolved in distilled water, and the solution made up accurately to 1 liter. Next 25 gms. of potassium iodide are dissolved in a little water, 12.7 gms. of iodine added, and the solution made up to about 900 or 950 c.c. To standardize this solution, 20 c.c. of the thiosulphate solution are carefully measured into a small flask, a few drops of the starch solution added, and then the iodine solution run in from a buret with glass stop-cock until the blue color just appears. This titration is repeated several times until the amount necessary for the end reaction is accurately determined. Then to the iodine solution is added the necessary amount of water so that 20 c.c. of this solution will exactly equal 20 c.c. of the thiosulphate solution and the resulting solution confirmed by another titration.

Both of these fluids are to be kept in dark glass bottles with ground-glass stoppers. The iodine solution must be restandardized frequently. One cubic centimeter of the thiosulphate solution equals 0.0127 gm. of iodine. The formula of the reaction is $2I+2Na_2S_2O_3=2NaI+Na_2S_4O_6$. The first trace of free iodine in excess will form the blue starch-iodine compound.

The urine if alkaline is first made just acid with acetic acid. To 500 c.c. of acid urine (if rich in acetone use 100 c.c. or even less) is added exactly 2 c.c. per 100 c.c. of the urine of 50% acetic acid. The urine is then distilled into a flask surrounded by ice and tightly closed by a stopper with two perforations. Through 1 of these passes the tube from the distilling flask, which tube reaches to the bottom of the flask and dips below the surface of water previously placed there; through the other perforation passes a shorter tube connected with a bulb or Peligot U-tube filled with water, which acts as a safety bulb to prevent the loss of any of the very volatile acetone. Then the urine is distilled until about 9/10 of its volume has passed over into the receiving flask. The distilling flask must be disconnected before the heat is removed, else the distillate will " strike back." The tube of the cooler is now washed thoroughly with distilled water over into the receiving flask so as to conserve the last trace of distillate. Lastly and for the same reason the water in the safety bulb or U-tube is emptied into, and its tube washed into, this flask.

Some calcium carbonate is then added to the distillate and the flask well shaken. This will remove any nitrous and formic acids which may have distilled over.

The distillation is now repeated as before. To this second distillate (to which again is added the water in the safety tube and the wash-water from both tubes) is added 1 c.c. of dilute sulphuric acid (1 : 8 of water), and the distillation again repeated, using the same precautions as before.

This final distillate is poured into a flask or measuring cylinder with ground-glass stopper and so large that the distillate and reagents next to be added will not fill it more than 1/3 full. (Or, this flask or cylinder may be used to receive the distillate during this distillation. It should of course be protected as in the first distillation.) A large excess of carefully measured *0.1N* iodine solution is added, these fluids well shaken, and then an excess of strong nitrate-free NaOH or KOH added drop by drop. The flask is closed, shaken for ¼ of a minute and then allowed to stand for 5 minutes.

The stopper is then removed (and any fluid clinging to it washed back into the flask), the contents of the flask are then made acid with concentrated hydrochloric acid and the excess of iodine determined by titration with the *0.1N* thiosulphate solution which should be added from a buret until the mixture is slightly yellow. Then a few cubic centimeters of the starch solution are added and the titration continued until the blue color has just disappeared. If one accidentally carries the titration too far he may add more of the iodine solution, carefully measured, and then continue the titration until the end reaction is reached. One cubic centimeter of the iodine solution indicates 0.967 mg. of acetone.

The results of this method are from 4 to 8% too low. The final distillate must contain no phenol, ammonia, nitrous or formic acids, for all of these but nitrous acid will cause the loss of some of the iodine, while nitrous acid will set iodine free. It is to prevent the error from ammonia that 2 c.c. of acetic acid per 100 c.c. of urine were added to the just-acid urine. If a mineral acid or as much as 5 c.c. of acetic per 100 c.c. of urine were used none of the ammonia would reach the distillate, but some phenol would. For this reason only 2 c.c. of acetic per 100 c.c. of urine are added and the trace of ammonia which does distil over is later removed by the third distillation after the addition of sulphuric acid. The addition of calcium carbonate to the first distillate will remove the nitrous and formic acids.

It is wise not to shake the final distillate when one adds to it the alkali and the iodine solution and to notice whether a black color appears at the line of separation of these 2 fluids. If it does, ammonia is present and the specimen should be thrown away. If not, the fluid is shaken, and one proceeds as above.

A much easier and fairly satisfactory method of determining the sum of acetone and diacetic acid in terms of acetone is the following:

From 50 to 250 c.c. of urine, according to the amount of acetone, to which is added a little acid to prevent foaming, is measured into a distilling flask. To the end of the outlet tube, which should pass through a very efficient cooler, is attached a rubber tube, the end of which dips beneath the surface of water previously placed in the receiving flask. The distillation is continued until most of the water has passed over.

The distillate is poured into a graduated cylinder with a ground-glass stopper, an excess (15 to 20 c.c.) of NaOH added, and then 20 c.c. of Lugol's solution, which may conveniently be made 3 times the ordinary strength. A heavy black precipitate forms which soon clears, leaving a yellow sediment of iodoform crystals. After standing for 10 to 15 minutes or more, about 40 to 50 c.c. of ether are added and the fluid well shaken until the ether contains practically all of the iodoform. After a reading is made of the volume of ether, 10 c.c. of it are measured with a graduated pipet into a weighed glass dish and evaporated in the air. This is then dried to constant weight over sulphuric acid. The weight of the iodoform multiplied by 0.147 equals the weight of acetone represented in the 10 c.c. of ether extract used. From this the amount in the entire volume of ether extract may be reckoned.

Folin's [80] *method* allows an estimation of the acetone alone. The acetone is separated from the urine by the same apparatus devised by Folin for ammonia estimations (see page 124). The urine, 25 c.c., is measured into the aërometer cylinder, and from 0.2 to 0.3 gm. of oxalic acid or a few drops of 10% phosphoric acid and 8 to 10 gms. of sodium chloride and a little petroleum added. In the receiving bottle has been previously placed water to which is added 40% potassium hydroxide solution (10 c.c. per 150 c.c. of the water) and an excess of the standardized iodine solution. The apparatus is then connected with a Chapman air pump and a fairly strong (yet not as strong as for an ammonia determination) air current drawn through for from 20 to 25 minutes. Every trace of acetone will be removed from the urine and converted in the receiving bottle to iodoform. The contents of the receiving bottle are acidified with concentrated hydrochloric acid (10 c.c. for each 10 c.c. of alkali used) and the excess of iodine titrated with standard thiosulphate and iodine solutions as in the Messinger method. The observer must be thoroughly acquainted with his apparatus and the strength of his air current by repeated experiments, using solutions to which known amounts of pure acetone have been added.

Diacetic Acid—Acetoacetic Acid, CH_3COCH_2COOH.—Diacetic acid is from the clinical viewpoint the most important of the acetone bodies, since it not only is the easiest to test for, but also is a very important index of acidosis. The present idea is that all diacetic acid in the urine is derived from β-oxybutyric acid, and all the acetone in turn from diacetic acid. The urine of a normal person should contain but a trace, if any, of diacetic acid and probably none if on a mixed diet. It appears quite early in the urine of a person starving, or on a diet poor in carbohydrates, and will promptly disappear if even a little carbohydrate be added to the diet. But individual differences in the output of this acid are so great that some other factors must be more important than the mere lack of carbohydrate in the diet. The statement is often made that one finds diacetic acid in those urines only which contain acetone in large amounts and not always then, yet the work of Folin would seem to prove that acetone is never present in such urines in more than traces, and that the tests used for acetone show in fact diacetic acid.

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[80] The Jour. of Biol. Chem., May, 1907.

Diacetic acid is found in the urine in increased amounts in the same conditions mentioned in page 187 for acetone. That is, in diabetes mellitus, in conditions of undernutrition or with defective absorption from the bowel, and hence in all cachexia-producing diseases; and in fevers, even in mild cases, especially the acute exanthemata of children during the eruptive stage and still more especially in streptococcus infections. Its amount would seem to depend much on the nature of the infection. Its presence is not limited to the febrile periods and it has no prognostic importance. Next to diabetes the most important group of cases with diacetic acid in the urine is that of gastro-intestinal disturbances. In these cases its presence is by no means explained by the lack of carbohydrate, but would seem to depend on abnormal fat catabolism. It is found in very mild cases; it does not disappear when sugar is added to the diet as promptly as it does from the urine of a normal person on a pure proteid diet. It is said to occur in especially large amounts in the urine of drunkards with gastro-intestinal disturbances. Rolleston and Tebbs [81] found it present in abundance in 33 of 38 cases of gastric ulcer treated either by starvation or by rectal feeding. The tests for it became positive in from 2 to 12 days, usually 1 or 2 days, after treatment began and disappeared in from 1 to 14 days, usually 5, after the return to mouth feeding. Women would seem to excrete especially large amounts of this acid; age and the chronicity of the disease would seem of no moment in its production. In some of these cases as much ammonia (the best index of acids) is present in the urine as in diabetes (Golla). It may be found in the urine of normal men who for a few days have been on a pure proteid diet, and in mental cases who are losing weight and suffering from inanition.[82]

Gerhardt's Test.—The best test for diacetic acid is that proposed by Gerhardt. To from 10 to 50 c.c. of fresh urine are added a few drops of a Fe_2Cl_6 solution, which must not be too acid. This is added as long as a precipitate forms, and then the urine is filtered. To the filtrate is added still more Fe_2Cl_6. If diacetic acid is present the urine takes on a Bordeaux-red color, cherry-red by transmitted, purple-red by reflected, light. This test indicates from 0.4 to 0.5 p.m. of diacetic acid. Cyanates, NaAc, salicylic acid and its allied bodies, salol, aspirin, diuretin, and certain other medicines also will give a somewhat similar color, but with this difference that of all these substances diacetic acid is the only one which is destroyed by heat, hence a positive test should always be controlled by another made after the urine, first made weakly acid, has been boiled and then cooled. In this control test the red color of the urine should be distinctly paler than in the unboiled, since some at least of the diacetic acid will have been broken down. A modification of this test is to shake out the urine acidified with H_2SO_4 with ether and this with water, and to add the Fe_2Cl_6 solution to the water extract. A violet-red color appears in the water layer if this acid is present. This color pales on standing in from 24 to 48 hours, a necessary part of the reaction to exclude other bodies.

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[81] Brit. Med. Jour., 1904, vol. ii, p. 114.

[82] See also Futcher, Med. News, Oct. 8, 1904.

Arnold's Test.—Two solutions are kept in stock. One is a 1% aqueous solution of para-amido-acetophenon with 2 c.c. of strong hydrochloric acid in each 100 c.c. of the mixture; the other is a 1% solution of potassium or sodium nitrite. Two parts of the first and one of the second solution are mixed together in a test-tube, an equal bulk of urine added and finally a drop of strong ammonium hydrate. In normal urines a brown color usually appears which on the addition of several drops of strong hydrochloric acid changes to yellow, while in a urine containing diacetic acid a purple color develops. Normal urines may develop a red color similar to that given by urines containing very slight traces of diacetic acid, but on shaking the urine containing the diacetic acid the foam will show a violet color.

β-oxybutyric Acid, β-hydroxybutyric Acid, $CH_3CHOHCH_2COOH$.—Butyric acid is considered the mother substance of diacetic acid and hence of acetone. One might expect therefore to find it when they are present, especially if they are present in large amounts. Yet the chances are against finding it since it breaks up so readily to form diacetic acid. Gerhardt and Schlesinger[83] showed that it will appear in the urine of a normal man who has been for some days on a proteid diet (about 9 gms. of this acid were eliminated in 24 hours). This is the acid to which is attributed the acid intoxication (or alkali starvation) which is said to explain diabetic coma. Often about 50 gms. a day are excreted, and in one of Naunyn's cases 100 gms. a day for a long time. Larger amounts are excreted during coma: 188 gms. in 24 hours (Magnus-Levy); 225 gms. (Kulz) and in Joslin's case, 437 gms. in 3 days, *i.e.*, 3 gms. per 1 kilo per day.

Joslin believes that it is the alkaline treatment itself which explains these huge figures and cautions against the administration of any alkalies at all. Whether oxybutric acid has a specific toxicity or is toxic because it is an acid, is in doubt. The Strassburg school holds the latter view. On the other hand Wilbur[84] found that the injection into animals of this neutralized acid gave results similar to those of the free acid. He emphasized the point that the alkaline treatment of coma has not been as satisfactory as one would on theoretical grounds expect.

This acid is levorotatory, $[\alpha]D = -24.12°$. Its presence, therefore, may be suspected when the percentage of sugar measured with the polariscope is less than that indicated by titration. The presence of other levorotatory bodies, however, should always be considered: levulose, paired glycuronic acid compounds, albumin, etc.

Detection.—β-oxybutyric acid is probably present if the fermented urine of a diabetic shows a definite levorotation. It is quite certainly present if the ether extract of urine which has been fermented, then acidified with phosphoric acid and then extracted with ether, is levorotatory.

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[83] Arch. f. exp. Path. u. Pharm., 1898.

[84] Jour. Am. Med. Assoc., 1904, No. 17.

A very reliable test for this acid is the following: The urine is fermented till all glucose is gone. It is then evaporated to a syrup, an equal amount of concentrated H_2SO_4 added and then distilled. Crotonic acid is now present in the distillate which when cooled will separate in beautiful crystals with a melting point of 72° C. If these crystals do not readily form, the distillate may be shaken out with ether, the ether evaporated, the residue washed with water and then allowed to crystallize.

Quantitative Determination—*Black's Method.*—One measures 100 c.c. of urine with a pipet into an evaporating dish, makes it distinctly alkaline with sodium bicarbonate and then evaporates it to a thick syrupy liquid (4 or 5 c.c.), using the gentle heat of a water-bath or the low heat of an electric stove. This residue is cooled, made distinctly acid with strong hydrochloric acid and is then mixed gradually with plaster-of-Paris until it forms a porous, mealy mass. This porous meal is transferred to an extraction apparatus (that of Soxhlet or one of its modifications) and extracted for 3 hours with about 60 c.c. of ether. The ether extract is then transferred to an evaporating dish and the ether allowed to evaporate spontaneously. The residue is mixed with 5 c.c. of water, 0.4 gms. of bone-black added to decolorize it and it is then filtered and washed until perfectly clear. The filtrate is next made up to a known volume, usually 25 c.c., and the amount of β-hydroxybutyric acid which it contains determined with a polariscope, using the following formula:

$$\text{Grams of } \beta\text{-oxybutyric acid in 1 c.c.} = \frac{\text{Angle observed}}{24.12 \times 200}$$

24.12 = specific rotation of this acid
200 = length of polariscope tube.

Shaffer's Method for Determining β-hydroxybutyric Acid, Acetone and Diacetic Acid Combined.—One measures from 25 to 250 c.c. of the urine to be examined (estimating as nearly as possible that volume which would contain from 25 to 50 mgms. of acetone—from 25 to 50 c.c. of a urine which gives a strong ferric chloride reaction will suffice) and adds to it a definite excess of basic lead acetate and 10 c.c. of ammonium hydroxide. The mixture is made up to the 500 c.c. mark, shaken thoroughly and filtered through a dry filter paper. To 200 c.c. of the filtrate, measured into a distilling apparatus, are added from 300 to 400 c.c. of water, 15 c.c. of concentrated sulphuric acid and a little talcum to prevent knocking, and the distillation continued until from 200 to 250 c.c. of distillate have collected. This distillate contains, in addition to the preformed acetone, that which results from the breaking down of diacetic acid and certain volatile acids. It is therefore made slightly alkaline and distilled a second time. This distillate will contain all of the acetone (that originally preformed and that from the diacetic acid) and this may be estimated by the iodine method.

The residue containing the sulphuric acid contains all of the β-hydroxybutyric acid, which now must be converted by oxidation into acetone. The residue is therefore diluted, 0.5 gm. of potassium dichromate added and then distilled from a flask provided with a dropping funnel through which water is added gradually to make up for the amount distilled over. Instead of water one may use a 0.5% solution of potassium dichromate. To about 500 c.c. of the distillate are added 20 c.c. of 3% hydrogen dioxide, sufficient potassium hydroxide to make the solution alkaline and the distillate is redistilled until about 300 c.c. have passed over. The amount of acetone in this distillate is estimated by the iodine method. One milligram of acetone is equivalent to 1.794 mgms. of β-hydroxybutyric acid.

Van Slyke-Fitz Methods for the Determination of β-hydroxybutyric Acid, Diacetic Acid and Acetone in Urine and Blood (quoted from Joslin)—*Solutions Required.*—A 20% copper sulphate solution prepared by dissolving 200 gms. of $CuSO_4 5H_2O$ in water and making the solution up to 1 liter.

A 10% mercuric sulphate solution prepared by dissolving 73 gms. of chemically pure red mercuric oxide in 1 liter of *4N* H_2SO_4. The solution of the oxide is assisted by warming it on a steam-bath.

Fifty volume per cent. sulphuric acid prepared by diluting 500 c.c. of H_2SO_4 (sp. gr. 1.835) to 1 liter with water. This should be *17N* H_2SO_4.

Colloidal iron; Merck's "dialyzed iron" solution, containing 5% of Fe_2O_3.

Ten per cent. calcium hydrate suspension containing 100 gms. of Merck's fine light "reagent" $Ca(OH)_2$ mixed with 1 liter of water.

Five per cent. potassium dichromate solution prepared by dissolving 50 gms. of $K_2Cr_2O_7$ in 1 liter of water.

To remove glucose and other substances which would interfere, 25 c.c. of the urine to be examined are measured into a 250 c.c. measuring flask, 100 c.c. of water and 50 c.c. of the copper sulphate solution added and the solutions well mixed. To this are then added 50 c.c. of 10% calcium hydrate. The solution is well shaken and tested with litmus. If it is not alkaline in reaction more calcium hydrate is added. The solution is then diluted to the mark and let stand for at least ½ hour to allow the glucose to precipitate. It is then filtered through a dry folded filter. This procedure will remove all the glucose of an 8% or weaker solution, therefore urine containing more should be so diluted that the percentage of glucose may be less than 8%. To be sure that the filtrate is free from glucose a little is boiled in a test-tube. A yellow precipitate (of Cu_2O) will indicate glucose; a white precipitate (of $CaCO_3$) has no significance.

To remove all proteins from the blood 10 c.c. of whole blood are measured into a 250 c.c. volumetric flask half full of water, 50 c.c. of colloidal iron are added, this mixed, then 1 c.c. of the saturated sodium sulphate solution added. The flask is then filled to the mark, shaken and the contents filtered through a dry folded filter.

If plasma is to be examined the procedure is the same except that only 8 c.c. are measured in a 200 c.c. flask and 15 c.c. of colloidal iron and 1 c.c. of saturated sodium sulphate are added.

In the case of either whole blood or serum 125 c.c. of the filtrate, equivalent to 5 c.c. of the original sample, are taken for analysis.

Simultaneous determination of total acetone bodies (acetone, diacetic acid and β-hydroxybutyric acid) of the urine or blood in one operation. Into a 500 c.c. Erlenmeyer flask are measured 25 c.c. of the urine filtrate plus 100 c.c. of water; or 125 c.c. of the blood filtrate plus 10 c.c. of 50% sulphuric acid and 35 c.c. of the 10% mercuric sulphate solution. The flask is then connected with a reflux condenser having a straight condensing tube of 8 or 10 mm. diameter and heated to boiling. After the boiling has begun, 5 c.c. of the 5% dichromate solution are added through the condenser tube and the boiling continued gently for 1½ hours. The precipitate which forms consists of the mercury sulphate compound of acetone, both that preformed and that formed by the oxidation of the hydroxybutyric acid. The precipitate is collected into a Gooch, or a medium density "alundum," crucible, washed with 200 c.c. of cold water followed by a little 95% alcohol, dried for an hour at 110° and weighed. Several precipitates may be collected, one above the other, without cleaning the crucible.

Acetone and Diacetic Acid.—These substances without the hydroxybutyric acid are determined exactly as are the total acetone bodies, except that (1) no dichromate is added to oxidize the hydroxybutyric acid and (2) the boiling must continue for not less than 35 nor more than 45 minutes. Boiling for more than 45 minutes would split off a little acetone from hydroxybutyric acid even though no dichromate had been added.

β-hydroxvbutyric Acid in Urine.—The β-hydroxybutyric acid alone is determined exactly as total acetone bodies except that the preformed acetone and that formed from the diacetic acid are first boiled off. To do this the 25 c.c. of urine filtrate plus 125 c.c. of water are treated with 2 c.c. of 50% sulphuric acid and boiled in the open flask for 10 minutes. The volume of solution left in the flask is measured in a cylinder, then returned to the flask and the cylinder washed with enough water to replace part of that boiled off and to bring the volume of the solution to 127 c.c. Then 8 c.c. of the 50% acid and 35 c.c. of the 10% mercuric sulphate solution are added. The flask is connected under the condenser and the determination is continued as above.

β-hydroxybutyric Acid in Blood.—The following procedure enables one to determine separately in a single sample of blood both the acetone plus the diacetic acid and the hydroxybutyric acid. The acetone and diacetic acid are precipitated as above described and the filtrate poured as completely as possible through the Gooch or alundum crucible into a dry receiving flask. Of this filtrate 160 c.c. are measured into another Erlenmeyer flask and 10 c.c. of water are added. The mixture is heated to boiling under a reflux condenser, 5 c.c. of dichromate solution are added and the determination continued as described for "total acetone bodies."

In case only the β-acid is to be estimated, or if enough blood is taken for 2 determinations, the slightly easier procedure used for urine may be followed also with blood.

Factors for calculating the acetone bodies in urine, when 25 c.c. of the filtrate, equivalent to 2.5 c.c. of urine, and in blood when 135 c.c. of filtrate, equivalent to 5 c.c. of blood, are used for determination. One milligram of β-hydroxybutyric acid yields 8.7 mgms. of precipitate. One milligram of acetone yields 19.7 mgms. of precipitate.

The amount of precipitate obtained from β-hydroxybutyric acid therefore corresponds to 79% of the acetone that would be obtained if each molecule of hydroxybutyric yielded a molecule of acetone. The oxidation is complete in 1½ hours and the conditions are so constant that duplicates usually check within 1%.

DIABETES MELLITUS

Diabetes mellitus is a disease the most important characteristic of which is a reduction of the ability of the tissue cells to use the glucose molecule. So far as we know all cases of long-standing (*i.e.*, more than 1 week) glycosuria are cases of diabetes mellitus, but the urine of a patient with diabetes mellitus may be sugar-free even for months, provided the carbohydrates in the diet do not exceed the limit of ability of the body to warehouse glucose. Indeed a case of diabetes mellitus may theoretically during his life never be a case of glycosuria except during the periods when his sugar tolerance is being tested. The kidneys and therefore the urine are only secondarily involved in this disease. The lessened ability of the tissue-cells to burn glucose would seem to depend either on a limitation of the supply of some substance in the blood which like a key is necessary to unlock the glucose molecules (although their ability to burn any other molecule may be quite unimpaired) or of a substance which when in combination with glucose renders this suitable for use by the tissue cells. This missing substance has been called the "internal secretion," the "pancreatic amboceptor," etc. The student should bear in mind that in a well marked case of diabetes mellitus we are dealing with at least 6 different disease complexes, and that these while related are nevertheless in many particulars independent: first, the systemic disease, which in diabetes happens to affect the pancreas especially, *e.g.*, cancer, lues, etc., diseases which apart from their relation to the pancreas have their own pathology and prognosis; second, this disease of the pancreas itself; third, the glycosuria; fourth, the production of abnormal bodies, many of them acid in nature, evidently products of the incomplete combustion of fats and proteids; fifth, conditions arising from the alkali starvation produced by the presence and elimination of these acid bodies; and sixth, various complications and sequelæ, as gangrene, degenerations in the central nervous system and peripheral nerves, pyogenic infections favored by the lowered resistance of the tissues due to the diabetic condition, etc. Each one of this group of conditions gives rise to problems in diagnosis peculiar to itself.

Cases of diabetes mellitus may be classified as severe, if they have a tolerance of from 0 to 10 gms. of carbohydrate; moderate, if they have a tolerance of from 10 to 50 gms. and mild if the tolerance is over 50 gms. Since practically every case may be made sugar-free by proper fasting and diet, the older definition of severe cases as those who cannot be made sugar-free on a carbohydrate-free diet can no longer be used. How severe a case may be can often be determined only after long observation and will depend less on the history of his glycosuria than on the prognosis of his underlying diseases.

This is well illustrated by the case of Geyelin and Dubois [85] who was admitted with symptoms of most severe diabetes mellitus of 6 weeks duration and bordering on diabetic coma and discharged 3 months later, a mild case.

The urine in diabetes mellitus is, as a rule, but not necessarily, increased in amount. This increase is often not marked unless the urine contains over from 2 to 3% of sugar, beyond which point the volume is roughly proportional to the amount of glucose present. Severe cases, that is, those excreting 5% or more of sugar, may void from 4 to 5 liters, even 10 liters, while there is 1 case on record who voided 28 liters of urine in 24 hours. Joslin's record case was a boy 10 years old who weighed 18.6 kilos and who in 16 hours voided 7200 c.c. of urine or 39% of his weight. On the other hand some patients void small volumes of urine with a high percentage of sugar. This is considered to indicate a good prognosis although a polyuria does not indicate a bad one. Naunyn reported 2 such cases, 1 with 1400 c.c. of urine containing 9% of sugar and with a specific gravity of 1.040; a second with 2800 c.c., 10.5% of sugar and specific gravity 1.047. Joslin mentions a case with 1035 c.c. of urine in 24 hours and 5.8% of sugar. In other cases the reverse is true and there is a polyuria with a low percentage of glucose, but this is rare except in those cases in which an increasing acidosis accompanies a decreasing glycosuria and especially in cases which follow injury to the skull. In one such case the specific gravity was 1.003 and sugar 1% (Naunyn's case). A similar condition may be met with in a diabetic who is developing chronic interstitial nephritis and in some cases of diabetes with marked asthenia.

It is in diabetes mellitus that the specific gravity of the urine reaches record figures. As a rule it ranges between 1.030 and 1.040. In one of

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[85] Jour. A. M. A., 1916, vol. 66, p. 1532.

Naunyn's cases it was 1.060, and he mentions one reported in which it was 1.074. A polyuria is usually present if the urine in a case of this form of diabetes has a specific gravity of 1.030. This is the one condition in which there is both a high specific gravity and an increased amount of urine, but the specific gravity bears little relation to the latter.

The chief sugar present is glucose, yet levulose, pentose and other carbohydrates may also be present; in rare cases levulose alone is found. There is an increase also of the unfermentable carbohydrates [a minimum of 20 gms. instead of 1.6 gms. (normal maximum, 5 gms.) per day].[86]

The urine has a suggestive pale greenish-yellow color. It will ferment spontaneously, with the evolution of CO_2 and the deposit of a sediment. This fermentation may take place in the bladder and consume the sugar so that a sugar-free urine may be excreted. Again, the sugar may disappear by a fermentation without gas production.

In testing the urine qualitatively for glucose it is sometimes important to choose the right specimen. If sugar is present in all voidings this is not important, but mild cases may eliminate little sugar and that only during a few hours of each day. Did we examine this one voiding there would be no doubt as to the presence of sugar, but if this voiding is mixed with all the other voidings of that day the solution of glucose may be so dilute that it will not give a positive test. For that reason we examine that specimen of urine voided from 4 to 6 hours after a noon meal which contains some carbohydrate. The maximum of sugar excretion comes late in the forenoon, even though the ingestion of carbohydrates extends equally over the whole day. There is another maximum, a somewhat less one, in the late afternoon (about 6 o'clock). The minimal excretion is early in the morning. In some severe cases the variations in sugar output are but little marked and in still more severe cases much more may be excreted during the night than during the day (note the resemblance to the excretion of water and solids in nephritis). In mild cases on a mixed diet the urine may be sugar-free during the night and reach even 3% during the day. In some cases sugar-free periods lasting for months will alternate with periods of glycosuria. It is thus evident that mild cases may easily be overlooked if but one specimen of urine is examined. The output of sugar is greater in hot than in cold weather. This is also true of the carbohydrate of normal urines. Cases of diabetes mellitus have been classified according to their percentage of sugar excretion (their "intensity") and also according to the total amount of sugar excreted in a day (or their "size"). Cases can be compared in this way only if they are on a constant diet and such a classification has but little value. The amount of sugar eliminated per day by severe cases on a liberal diet is often 800 gms. and in one case 1500 gms. in 24 hours. These same patients on a more limited diet seldom excrete over 200 gms. Starving patients are usually sugar-free.

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[86] Edsall, Am. Jour. Med. Sci., 1901.

As regards the relation between water and glucose excretion, it may be said that diabetics responded to an increased intake of fluid more slowly than do normal people. In glucose the water excretion depends in large measure upon the amount of glucose to be eliminated although this does not explain the day and night variations.

Influence of Diet.—The sugar output is increased by a carbohydrate diet, especially by dextrose and its polysaccharides. If a diabetic be fed levulose he will use it fairly well for a day or two and then excrete it as glucose. The starches of potato and oatmeal would seem to be very well borne and yet this relationship between diet and glycosuria is in some measure only apparent since the sugars differ much in the rate of their absorption and the ability of the body to store instead of to use them. For illustration, the " good " results observed of the potato and oatmeal " cures " were in part explained by the partial starvation of these badly fed patients and second by the ease with which these starches are digested by the external secretion of the pancreas which therefore is less fatigued and so better able to produce its internal secretion. In severe cases the variation in the sugar output depends in part on gastro-intestinal troubles so common in diabetes which may prevent the absorption of sugar. Muscle work, up to a certain point, decreases glycosuria, while psychical influences such as fright, mental strain, or worry will increase it much and may even bring a latent case to light. Hence in the treatment of diabetics moderate physical work and a peaceful mind are important points. Mendel and Lusk [87] found that in completely diabetic dogs on a constant proteid-fat diet the ratio of glucose to nitrogen in the urine was constantly 3.65 : 1. But no man yet tested has been found completely diabetic; that is, all can burn some glucose and certainly one is never justified in exposing his patient to the injury which the above diet test would entail. It is interesting that Geyalin and DuBois' case (see page 199) for a considerable period have had D : N = 3.6 : 1, *i.e.*, had Lusk's " fatal ratio " and yet improved.

Intensity of Glycosuria.—On a rich carbohydrate diet the percentage of glucose may reach, but rarely exceeds, 6 to 8%. Naunyn mentions a case with 11%, while others mention a case with 20% of glucose in the urine.

The effect of acute infections on a glycosuria is variable and often particularly interesting. In pneumonia, for instance, a remarkable diminution in the sugar output due to an increased tolerance to carbohydrates begins with the rise of temperature and is not due to the diet. No satisfactory explanation has been given. On the other hand the sugar output may be increased during a fever or a glycosuria may begin with and continue after a febrile disease. This is the reason why so many diseases which have been contracted by patients with latent glycosuria have been reported as the " cause " of diabetes mellitus.

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[87] Deutsches Arch. f. klin. Med., 1904, vol. lxxxi.

Chronic diseases such as tuberculosis of the lungs, diseases of the central nervous system, circulatory disturbances with albuminuria and nephritis tend to diminish the sugar output. As some diseases develop, and this is true of Bright's disease especially, the glycosuria progressively diminishes and finally disappears, hence they are reported as cured. This decrease in the glycosuria is due to a definite raising of the threshold point of the kidney for glucose and not to changes in the diet. Neither is it due to an increased inability of the kidneys to eliminate sugar since there is no increase in the hyperglycemia. An increase in the assimilation limit to glucose would seem to be a phenomenon of developing cachexia

The output of sugar in each case of diabetes is subject to spontaneous and wide fluctuations which must be due to variations in tolerance.

Severity and Tolerance.—A light case, according to Naunyn, is one which can eat daily 60 gms. of bread and remain sugar-free for a considerable time. Those persons are said to have a " paradoxical tolerance " who can consume daily considerable carbohydrate with only a trace of sugar in the urine and yet who cannot get rid of that last trace. These are severe cases. The student should remember, first, that a pure proteid diet is not sugar-free and second that while severe cases may keep sugar-free for some time on a full proteid-fat diet, yet it is at great expense to the body. There is a constant tendency for a large glycosuria to increase and the greater the glucose output the less becomes the tolerance. The slight glycosurias tend to diminish. A patient's tolerance suffers more from the ingestion of a large amount of glucose at one time than from the same amount in divided portions. The reverse is also true that tolerance is increased more by a brief period during which the patient is quite sugar-free than by a much longer period of slight glycosuria, hence the value of the " hunger day " of Naunyn and of the fasting periods of Allen. If as the result of fasting a patient becomes sugar-free for even 24 hours he will on the following day be able to use without the production of a glycosuria an amount of bread which previously would have caused a marked rise in the output of glucose. That is, the question of tolerance would seem closely related to that of the fatigue of a gland.

A case of " transitory diabetes " with acidosis is reported by Mann [38] which lasted 16 days, then disappeared even though the patient consumed much sugar.

Coma and Acidosis.—By " acidosis " Naunyn meant the accumulation in the body of those acid bodies which are formed in normal metabolism but which the healthy organism will rapidly eliminate, neutralize or further oxidize. The accumulation of these acid bodies produces an acid intoxication, or better expressed, an alkali starvation, and this it is believed is the explanation of diabetic coma. The laboratory evidence of acidosis includes the appearance in the urine of large amounts of acetone, diacetic acid, and, in severe cases, of β-hydroxybutyric acid, probably the mother substance of the two, a great increase in the output of ammonia which serves as a base for some of these acid bodies, a retention by the tissues of any alkali administered and a decrease of the CO_2 tension of the blood. The production in the body of these acetone bodies is not characteristic of a diabetic disturbance of metabolism, since the urine of a fasting person and of a normal person on a sugar-free diet will in 3 or more days contain them all. But in the diabetic these remain unoxidized and they may excrete from 20 to 30 gms. of oxybutyric acid daily for years. When an acidosis once begins the tendency is for it to increase. It is increased greatly by a rigid diet. This is the reason why the introduction of the strict dietary treatment of diabetes, the carbohydrate-free diet consisting of much proteid and fat, was followed by a great increase in the frequency of diabetic coma. As coma develops there is usually a sudden increase of these acid bodies in the urine. A patient may excrete daily for even months 20 gms. of oxybutyric acid, but if the output reaches 25 gms., on-coming coma should be feared (Herter). Any improvement in a patient in coma is followed by a greatly increased output of acid bodies, for it is not the acid in the urine which causes the trouble, but the acid which has not been excreted. Joslyn takes issue with this statement and feels that this increase is due to the administration of large amounts of alkali and is of no advantage to the body but rather is an injury. The presence of acidosis means that the case is a severe one, or at least that emaciation has begun, and yet such a patient may live for years. The amount of, or rather the danger from, these bodies has in the past been estimated by the ammonia output since the symptoms are caused especially by a withdrawal of the body alkalies which the ammonia protects. Any increase of ammonia output is said to mean the presence in the urine of at least 10 gms. of oxybutyric acid per day. A marked increase of ammonia indicates about 15 gms., while 4 gms. of ammonia per day indicates 16 gms. of the acid (Herter). Naunyn considers that the output of over 3 gms. of ammonia per day means danger of coma. Coma was the terminal event of 18 of Naunyn's 44 fatal cases, the most of them young persons from 21 to 30 years of age.

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[38] Berl. klin. Wochenschr., 1904, No. 30.

Sellards ("Acidosis," 1917) believes that for the determination of the degree of acidosis the titration of the blood is very inadequate, the methods of physical chemistry are of no clinical value, that Rountree's method is inaccurate, while the determination of urine ammonia is of no value since the ammonia may be increased in conditions without acidosis and may be normal or decreased when acidosis is present. He believes that Van Slyke's method of determining the carbon dioxide tension of the blood may be feasible. His own bicarbonate deficit test is, he believes, the most delicate of the tests which are specific for acidosis. By means of it one can detect very slight grades of experimental acidosis and moderate grades in diabetes before acetone appears or ammonia increases in the urine.

Sellards' Test.[89]—Normally 5 gms. of sodium bicarbonate administered by mouth will make the urine alkaline, but if the tissues have been depleted of their alkali, even 90 gms. may be injected intravenously without change of reaction of the urine. Such a retention is seen in diabetes mellitus, in the nephritis of cholera and lesser grades in chronic interstitial nephritis, in acute nephritis and in renal anteriosclerosis, but not in chronic parenchymatous nephritis.

Sellards sterilizes a 2% solution of sodium bicarbonate in an autoclave at a pressure of 10 pounds for 30 minutes in an atmosphere of CO_2 (generated by adding sodium bicarbonate to the water supplying the autoclave). The sterilized solution is kept in cork-stoppered bottles and is used not later than 2 or 3 days after sterilization. Small amounts may be administered by mouth but larger must be injected intravenously. The reaction of the urine is tested with litmus paper. If the urine is but slightly acid it should be boiled before it is tested. The urine is collected at 3 hour intervals.

Frothingham [90] using several methods, studied groups of cases of diabetes, syphilis, epilepsy, exophthalmic goiter, primary anemia, chronic nephritis, pneumonia, acute rheumatic fever, subacute nephritis, lung abscess, gastric ulcer, Addison's disease, cirrhosis of the liver, chronic cardiac disease, as well as single cases of 17 other conditions, found an acidosis in some cases of diabetes, chronic nephritis, pneumonia, acute articular rheumatism and in several acute febrile conditions in the miscellaneous group.

Acidosis and Uremia.—While a mild grade of acidosis may develop in cases of uncomplicated nephritis, yet this will be compensated for by the increased excretion of acids by the kidney until advanced uremia develops and then there may be accumulations of non-volatile acids in the blood sufficient to depress the tension of CO_2 of the alveolar air. This acidosis does not, however, run parallel to the nitrogen retention or the output of phenolsulphonephthalein.[91]

A sign of coma in diabetes always suggestive, though not conclusive, is the appearance in the urine of such large numbers of granular casts that they form a gross sediment. This may appear with the coma or give warning even 24 hours in advance (Külz's sign, page 272).

Among the other urinary symptoms in diabetes is an increase in the output of the creatinin (even 2 gms. per day), the animal gum of Landwehr (much), uric acid, phosphoric acid and sulphuric acid. In all of these conditions, however, the increase is due to the diet and not to the disease. Oxalic acid also is increased (even to 1.2 gms. per day), especially as the sugar disappears.

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[89] Bull. of the Johns Hopkins Hosp., Oct., 1912, vol. xxiii, p. 289.
[90] Arch. of Int. Med., Dec., 1916, vol. xviii, p. 1717.
[91] Peabody, Arch. of Int. Med., 1914, vol. 14, p. 236.

The urine of these patients often contains albumin. In some this is due to a complicating disease, but of Naunyn's cases of pure diabetes 32 of 94 had albuminuria. Of these in 17 there was only occasionally a trace of albumin present, in 6 the trace was almost constant, while in 10 there was marked albuminuria. Naunyn believes that an albuminuria may be an expression of the effect of a glycosuria on the kidney. On the other hand, it is interesting that as chronic nephritis develops a glycosuria gradually disappears. This explains many of the so-called " cures " of diabetes mellitus. In other cases a glycosuria and an albuminuria may alternate.

Diabetes Insipidus.—Diabetes insipidus is a disease of long duration the chief symptom of which is a marked polyuria of normal urine of very low specific gravity. It is a rare condition. Futcher reported from the Johns Hopkins Hospital clinic but 4 cases, or 0.001% of admissions. It occurs particularly in young men, although Jacobi considered that fully 25% of the cases are in children under 10 years of age.

The cases may be grouped as primary or idiopathic, which have no known lesion, and the secondary or symptomatic. The latest and best theory for the primary cases is that they are due to an increase in the secretion from the posterior and intermediate lobes of the pituitary gland. The secondary cases are associated with tumors of the brain, cerebral trauma, cerebral hemorrhage, cerebral lues especially and basilar meningitis, which diseases may of course affect the pituitary gland. Marked polyuria may be associated with certain diseases of the abdominal viscera and of the spinal cord, it is an occasional symptom of the psychoses, of hysteria, of epilepsy and of chorea. Some of these cases resemble closely diabetes insipidus, others suggest rather a primary polydipsia, since on limiting the intake of fluids the urine becomes almost normal in amount.

Polyuria is the most conspicuous symptom of this disease. From 20 to 40 (and in 1 case, 43) liters may be voided in 24 hours. The amount voided daily by two children was almost equal to their body weight. The urine is pale, watery in color, faintly acid in reaction, and with a specific gravity of from 1.002 to 1.005 or perhaps 1.010. Failure to make the necessary temperature correction probably explains some of the impossible figures of specific gravity reported. In a few cases the specific gravity of the urine, although there was polyuria, is more nearly normal (*e.g.*, 6 liters and 1.017). The urine of these cases shows a remarkable fixation of the specific gravity, *i.e.*, this remains almost constant in spite of all means taken to raise it (the ingestion of NaCl, etc.). Albuminuria and cylindruria are absent, or if present mean a complicating condition. Sugar is also absent and yet there are cases of diabetes insipidus which later develop into diabetes mellitus and vice versa. Brackett's [92] case began suddenly as polyuria following mental shock, but 7 months later, just before death, the specific gravity which had varied from 1.002 to 1.006 rose to 1.026 and considerable sugar appeared. Some patients with diabetes insipidus would seem to void daily an amount of urine greater than the fluid intake (including the water content of the solid foods). The urine voided by one of Futcher's cases, for instance, exceeded the fluid intake by from 400 to 6355 c.c. per day and yet this patient was carefully watched to prevent deception. The solids of the urine of a case of diabetes insipidus are increased because of the polyuria which " washes " these from the blood. This explains the remarkable polyphagia, as a result of which their urea output may reach 80 gms. or more per day. The output of sodium chloride and of phosphoric acid is either normal or slightly increased. Inosite is often present, probably washed out of the muscles by the unusual water flow due to the polydipsia since it can be found in the urine of normal persons after the ingestion of large amounts of water.

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[92] Lancet, 1899, No. 25.

Many believe that a condition of bradyuria exists in cases of diabetes insipidus; that is, that fluid ingested is more slowly eliminated than normal but this is not constant.

Glycuronic Acid, $CHO(CHOH)_4COOH$.—Glycuronic acid is an intermediate product of glucose metabolism which appears in the urine only when protected from further oxidation by conjunction with a suitable drug, as camphor, or with substances which arise in the body as indoxyl, skatoxyl, paracresol, phenol, or by combination with certain nitrogenous bases to form, *e.g.*, uramidoglycuronic acid. The amount excreted, normally less than 25 mgms. per 100 c.c. of urine, depends on the quantity of bodies present with which it can conjugate rather than on the amount of glycuronic acid formed. Free glycuronic acid crystallized with phenylhydrazine in beautiful needles whose melting point is 114° to 115° C. This acid, however, does not occur free in the urine, but must first be split from its paired compound by the addition, *e.g.*, of acid and heat. The glycuronates will reduce copper, bismuth and silver, reducing copper as readily as it does glucose. They do not ferment. With HCl and phloroglucin or orcin the free acid gives the same color tests as do the pentoses, including even their spectrum. The orcin reaction is the most convenient one to use (see page 185). It gives the furfurol test. While the free acid is dextrorotatory the paired compounds, which alone appear in the urine, all are levorotatory and explain the levorotation of from 0.05° to 0.17° of normal urine. The amount excreted is much increased by the administration of camphor and of chloral hydrate and by all conditions which increase the production of the substances mentioned above with which this acid can pair. The clinical importance of the glycuronic acid compounds lies in the fact that they occur in normal urine in amounts sufficient to reduce copper after somewhat prolonged boiling and are levorotatory. If, therefore, the reduction test for sugar is suggestive, the fermentation test negative and the orcin test positive, they should be suspected. Since this acid is a normal product of glucose oxidation, its elimination is increased in diabetes mellitus. Some, indeed, claim that in very mild cases of diabetes the urine contains increased amounts of this only and no glucose, but Edsall [93] showed that the excess of benzoyl esters in the urines of diabetics is not always due to an excess of glycuronic acid but rather to an increase of the unfermentable carbohydrates which are present in all intoxications and which may serve as a protective measure of the body to combat these intoxications, while Fisher has shown that the glycuronic acid is paired before any oxidation of the dextrose molecule has occurred.

Alkaptonuria.—That alkaptonuria is a rare condition is evident from the fact that but 40 cases (29 of them men) had been reported up to 1902 (Garrod) of which only 4 were in America (Futcher), and that this number has not increased much since attention was called to the condition. The condition causes no symptoms and is discovered accidentally, by the mother, for instance, noticing that the napkins of the infant are darkly stained, or by an insurance company examining carefully an applicant whose urine seems to contain sugar. Urine supposed to be rich in pyrocatechin often proves to contain alkapton bodies. Alkaptonuria seems to be a congenital and life-long variant or freak of metabolism although some cases are intermittant, and Mittelbach's patient is confident that his followed an injury. The explanation given is that the body is unable to burn homogentisinic acid, an intermediate product of proteid catabolism.[94] The condition is, therefore, comparable to glycosuria. Tyrosin, while the mother substance of some, cannot explain all of this acid in the urine.

Alkaptonuria would seem to be a family disease, since 19 of 32 cases occurred in 7 families, in 1 of which were 4 cases. There is only 1 case indicating inheritance (Osler's case).[95] Garrod [96] finds that 60% of the cases are children of parents who are first cousins. Others think that it is due to an intestinal mycosis, a peculiar intestinal ferment, etc. The amount of reducing substance excreted in the urine varies from about 3.2 to 6.9 gms. in 24 hours. It is of interest, said Garrod, that the output in each case is fairly constant and that each person excretes either the amount peculiar to himself or none. No traces, no gradual increases or decreases in the output are seen. Others find that the amount eliminated depends somewhat upon the diet; that it is reduced a little by a vegetable diet and to about one-half by starvation. Mittelbach claimed that the maximum excretion is from 1 to 3 hours after a heavy meal, which would indicate an intestinal origin, but Garrod found it to be from 4 to 8 hours after a meal, which would indicate a disturbance of tissue metabolism as the cause.

The urine of a patient with alkaptonuria when fresh is very acid in reaction and normal in color, but it rapidly becomes dark, reddish-brown and syrupy from oxidation, especially if the urine is made alkaline. It gives the copper test of glucose, but not Nylander's. $AgNO_3$ is reduced in the cold. It does not rotate polarized light, is not fermented and gives no crystals with phenylhydrazin.

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[93] Univ. of Penn. Med. Bull., April, 1906.

[94] See Langstein and Meyer, Deutsches Arch. f. klin. Med., 1903, vol. lxxviii, p. 161.

[95] Lancet, Jan. 2, 1904.

[96] Lancet, Dec. 13, 1902.

Of the alkapton bodies, 2 have been isolated; homogentisinic and uroleucinic acids. There may be others. V. Jaksch includes the glycosuric acid of Marshall, which may, however, for the most part be homogentisinic acid.

Homogentisinic acid $C_6H_3(OH)_2COOH$, is the most important and in most cases the only alkapton body present. This is the substance which explains the characteristic reactions of the urine. Its mother substance would seem to be tyrosin, for this, if fed a patient in small doses, is excreted as this acid.

To isolate homogentisinic acid the urine is made strongly acid with H_2SO_4 (1 to 12), 75 c.c. per 1 liter of urine. This is evaporated on a water bath to 1/10 its volume and shaken out 4 or 5 times with 3 volumes of ether. The ether is then distilled off, the residue dissolved in water (30 to 60 volumes), filtered, the solution heated to boiling and precipitated with 20% PbAc. This is quickly filtered while hot to separate the brown resinous precipitate. On standing the lead salts will slowly separate out. These are decomposed by H_2S, and the filtrate carefully evaporated first on the water-bath and then in vacuo. The acid will crystallize out. Garrod recommends that the urine be heated to boiling and from 5 to 6 gms. of solid PbAc per 100 c.c. of urine added. When this is dissolved the urine is filtered and the filtrate allowed to stand 24 hours in a cool place. The lead crystals which separate out are ground fine, suspended in water, decomposed with H_2S, filtered, evaporated first on the water-bath and then in vacuo to a syrup.

Uroleucinic acid, $C_6H_3(OH)_2C_2H_3OHCOOH$ (?) also has been found in the urine of patients with alkaptonuria. Its reactions are very similar to those of homogentisinic acid from which it may be separated since it is precipitated by basic lead acetate. Garrod found none in the urine of some in whom years previously others had found it.

Baumann's Quantitative Determination of Homogentisinic Acid.[97]—To 10 c.c. of the urine in a flask are added 10 c.c. of 3% ammonia. Then one adds at once several cubic centimeters of *0.1N* $AgNO_3$, shakes it a little and allows it to stand 5 minutes. Five drops of 10% $CaCl_2$ and 10 drops of ammonium carbonate solution are then added. After shaking, this is filtered. The brown-colored but entirely clear filtrate is tested with silver nitrate. If there at once appears a marked precipitate of reduced silver, the test is repeated, but a larger amount, even twice as much, of silver solution is added to the mixture of 10 c.c. of urine and 10 c.c. of ammonia. In this way one estimates approximately the amount of the silver solution necessary to oxidize the homogentisinic acid present. The end reaction is now determined by adding HCl. One is near this when on the addition of HCl the deep brown fluid takes a light red color. The end reaction is reached when the filtrate from the silver precipitate acidified with dilute HCl shows a slight precipitate of AgCl. One can determine this point very sharply by repeating the determination 4 or 6 times. If more than 8 c.c. of silver solution are necessary, on repeating the determination 20 c.c. rather than 10 c.c. of ammonia should be used.

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[97] Zeitschr. f. physiol. Chem., 1892, vol. xvi, p. 270.

One gram of the water-free homogentisinic acid is reduced with the above technic by a quantity of silver solution which contains 2.6 to 2.65 gms. of silver; that is, 240 to 254 c.c. of the *0.1N* silver solution. Hence 1 c.c. of the *0.1N* solution indicates 0.004124 gms. of the acid. The method has an average error of 6.1%. It is therefore only approximate.

Fat in the Urine (**Lipuria**).—The urine of the normal person contains practically no free fat. The scum of calcium phosphate on the surface of some urines may closely resemble fat, and oil used in catheterization must of course be excluded. Lipuria may accompany a diet too rich in fat, fat medication, prolonged suppuration, phosphorus poisoning or diabetic lipemia. In urines containing a cell-rich exudate of pus-cells (as in pyonephrosis), epithelial cells and epithelial and fatty casts, considerable fat may be liberated by the disintegration of these formed elements. In some cases, however, of chyluria the urine is turbid with fat droplets. In these cases there are usually ruptured varicosed lymphatic vessels on the wall of bladder or in the kidney. Since lymph has a very variable fat content the term lymphuria has been used of cases in which the lymph which mixes with the urine is poor in fat and chyluria if it is rich in fat. If, however, the lymph originates in the varicosed renal lymphatics the term lymphuria should be used, but if it is the contents of the thoracic duct, chyluria. Some of these cases are due to the blocking of the pelvic lymphatics or those of the pelvis of the kidney by the adult filaria worms. Other cases probably of nonparasitic origin are due to old abscesses which have established such anastomosis.

The urine in chyluria often resembles milk or is opaque and red from the presence of blood also (hematochyluria) and sometimes coagulates into a jelly-like mass.

In cases of chyluria the fat content of the urine varies remarkably (from 0 to 1.4%) with, although not in direct proportion to, the fat content of the ingested food.

To determine [98] the fat of the urine, 10 c.c. of the specimen to be examined are mixed with about an equal amount of sand and evaporated to dryness on the water bath. The sand containing the urinary residue is then collected in filter papers and extracted with ether for 12 hours in a Soxhlet fat extractor. The ether extract is then transferred to a weighed dish and the ether evaporated by a current of air until the dish has assumed a constant weight. From the amount found in this way the amount of ether-soluble fat in the total specimen of urine is calculated.

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[98] Carter, Arch. of Int. Med., 1916, xviii, p. 541.

PROTEIDS IN THE URINE

Tests for Albumin.—Urine to be tested for albumin should if possible be fresh but at least should always be clear. If it cannot be examined while fresh it should be protected from decomposition (see page 88), otherwise the rapid changes in its reaction may render the tests difficult. A turbid urine, the turbidity not due to bacteria, is best cleared by filtration through several thicknesses of paper. But when bacteria are present it is best filtered through infusorial earth, magnesia, or an asbestos filter although the infusorial earth may remove some of the albumin. A concentrated urine should always be diluted, since this will render albumin tests more sensitive rather than less so.

Hallauer's[99] work emphasizes the importance of diluting the urine. If a normal urine be concentrated by heat to ½ its volume and then serum albumin added, the heat and acetic acid test will be sensitive, but the heat and nitric acid, Heller's and the potassium ferrocyanide tests all will be negative. If the urine be concentrated to ¼ its volume and serum albumin added, none of these tests will show the albumin, and yet if we now dilute this specimen to its original volume, all of these tests will be positive. The potassium ferrocyanide test is the first to fail to show albumin, giving negative results after the specific gravity has reached about 1.030. It is the urea and the inorganic salts, especially phosphates, which inhibit the albumin tests.

The specimen of urine to be examined should be chosen with care, since albumin may be present only during a few hours of the day. In doubtful cases therefore it is well to examine an afternoon specimen voided an hour or so after active exercise. This may be clearly positive for albumin although were that voiding mixed with the other albumin-free voidings of that same day the test might be negative.

THE HEAT AND ACETIC ACID TEST.—A clean test-tube is filled to within an inch of the top with filtered, clear urine. Holding the tube by its lower end, one heats its upper half to the boiling point and then holding the tube against a black background examines the boiled urine for a cloud. For careful work an alcohol flame is the best heat since the gas flame may deposit on the glass a faint film which suggests albumin. If turbidity appears in the urine it may be a cloud of albumin or one of calcium phosphate and calcium carbonate. To rule out the latter, a few drops of 5% acetic acid are added until the urine is distinctly acid. The cloud of phosphates and carbonates will promptly disappear, the carbonates with effervescence. Instead of acetic acid Hammarsten recommends from 1 to 3 drops of a 25% HCl per 10 c.c. of urine. After the addition of each drop of acid the boiling should be repeated. The acid will render an albumin cloud more distinct and more flocculent.

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[99] Münch. med. Wchschr., 1903, p. 1539.

If the heat does not produce a cloud the acid should nevertheless be added, since the urine may not be acid enough to permit the albumin to coagulate. And, even though the boiled and acidified and again boiled urine remains perfectly clear, it may nevertheless contain a demonstrable amount of albumin which will appear as a distinct cloud if the tube be allowed to stand for about 15 minutes. It is the failure to observe this precaution which leads to most mistakes. It is said that some very acid albuminous urines are not clouded by boiling unless a drop of alkali be added. Acetic acid in excess does not produce soluble acid albumin. This is, we believe, the most reliable of all the routine tests for albumin and yet it may be improved by a slight modification.[100] To the urine is added ⅕ its volume of a saturated aqueous solution of sodium chloride and from 3 to 5 drops of 50% acetic acid. One then proceeds in the manner described above. The sodium chloride renders the test more sensitive and holds all nucleo-albumin in solution.

The more chronic the nephritis the whiter the albumin cloud. The more acute, the browner it is.

Another coagulum which may appear in urine thus tested is the so-called "nucleo-albumin" or mucin, but this would also be precipitated in the cold by acetic acid (see page 220). One should therefore always make a control test by adding the same amount of acetic acid to a similar amount of unheated urine. The urine gives a better test for nucleo-albumin if diluted; this precipitate is soluble in an excess of acid.

Resinous acids in the urine may lead to error if a great excess of acetic acid is added. This cloud is soluble in alcohol. This precipitate may be met with after the ingestion of certain resinous bodies: turpentine, benzoin, copaiba, balsam of Peru, tolu, cubebs, etc.

Heat and Nitric Acid.—This test differs from the above in that concentrated HNO_3 is used instead of dilute acetic acid. The HNO_3 should be used in fair excess since the danger is that too little rather than too much may be added. Hammerstein recommends to add 1 to 2 drops of 25% HNO_3 for each 1 c.c. of urine. The urine should be boiled before and after each addition of acid. A flocculent precipitate obtained under these conditions indicates albumin, since the phosphate clouds are dissolved and nucleo-albumin is soluble in this excess of HNO_3. A cloud which either appears or increases after the acidified and boiled urine is allowed to cool is of "albumose." Uric acid may precipitate when the urine cools but this cloud is granular and colored. To demonstrate a faint trace of albumin by this test the saturated chloride mixture should be added to urines of low specific value (see above). Since a very great excess of HNO_3 may dissolve a mere trace of albumin, the acid should be added gradually and the boiling repeated after each addition. In this test also the coagulation of a trace may not be evident until the specimen has stood for some time, then a coagulum would if present be found at the bottom of the tube.

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[100] See Hasting, Medical Record, July 7, 1906.

This test excludes the "albumin normally present," the "nucleoalbumin," and indicates the nature of "albumose" (Bence-Jones body) if present. The urates may deceive one if the urine is concentrated, but this precipitate is never flocculent; the same is true of the resinous acids. Biliverdin and other pigments may cause a cloud but this is soluble in alcohol.

The heat and acid tests are very delicate, indicating as they do 0.005 gm. of albumin per 100 c.c. of urine. They should, however, always be confirmed and they do not always indicate the albumoses.

HELLER'S NITRIC ACID TEST.—This is a contact test between urine and cold pure nitric acid. If albumin is present a line of precipitated acid albumin, insoluble in a fair excess (but soluble in a great excess) of the acid, will form at the plane of contact. This test has one advantage that no heat is necessary. Of all the mineral acids which have been used, nitric required the least acid per molecule of albumin to give an insoluble compound. This test is delicate, indicating, some say, 0.007% and others, even 0.002% (Hammarsten) of albumin. The best vessel in which to perform the contact test is a very large test-tube or a wineglass, or best of all a U-shaped tube like that pictured below (see Fig. 34).

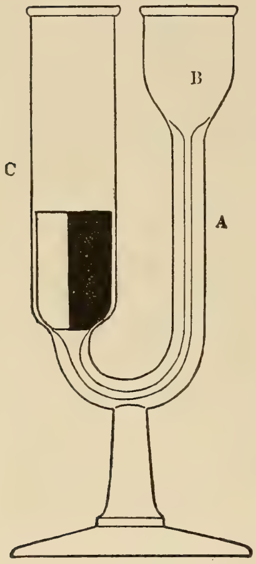

FIG. 34.—Horismascope. *A*, the arm of the U-shaped tube with fine bore; *B*, bulb in which HNO_3 is poured after the tubes are filled with urine; *C*, wide-bore arm for urine, with background.

As usually applied one pours into the tube about 2 inches of urine and then, holding the tube as horizontal as possible, allows the concentrated nitric acid to run slowly, best from a pipet, down the bottom of the tube. The object is to underlay 3 volumes of urine with 1 of HNO_3 and mix the fluids as little as possible. Some pour the urine in over the nitric acid. The nitric acid must be colorless, *i.e.*, contain no nitrous acid, since the effervescence which this and the urea at the line of contact will produce would prevent the formation of the ring and so a faint trace of albumin would go unrecognized. The same would be true if much carbonate is present, as in an old urine. The ring may not appear for several minutes. To see the faint line indicating a trace of albumin one should hold the tube against a dark background. If the ring appears at the end of 3 minutes the albumin content is less than 0.003%. This precipitate of acid albumin will develop exactly at the plane of contact; its thinness and density depend on the amount of albumin present and also on the skill with which the two fluids have been superimposed.

A red or reddish-violet ring at the plane of contact appears if an albumin-free urine is concentrated. This should not deceive one since it contains no precipitate.

A ring of precipitated urates often appears if the urine is concentrated but this is always above the line of contact and separated from it by a layer of clear urine. It also is broader than the albumin ring, is less distinct, it disappears on warming and will not appear if the test is repeated after the urine has been diluted with about 2 volumes of water, which dilution would often improve the albumin test. Sometimes, although rarely, this precipitate is very dense, clouding the whole volume of urine. Since this will disappear on warming and reappear on cooling it has been mistaken for the Bence-Jones body and a hopeless prognosis given.

Nucleo-albumin.—The body called often nucleo-albumin may give an opalescent ring 0.5 to 1 cm. above the line of contact and sometimes extending down to it. But this will disappear or at least move up (since the ring below disappears and a new ring forms above) if the tube is slightly shaken, since it is soluble in nitric acid. This ring may appear after the tube has stood for some minutes, it is faint and does not resemble the albumin ring much in appearance. If the urine is diluted and the test repeated, this precipitate will appear more rapidly and be more distinct.

Resinous Acids.—Heller's test applied to a urine containing resinous acids may produce a whitish ring above the line of contact of acid and urine which partly clears on warming. Since this precipitate is soluble in ether, to exclude the resinous acids one may pipet off the turbid layer of urine and mix it with a great excess of ether to prevent an emulsion. Or, one adds 2 or 3 drops of HCl to from 8 to 10 c.c. of the cold urine which will precipitate these acids. If then one adds more HCl and heats the urine, a red color will result. To remove these resinous acids from the urine one makes it strongly acid with acetic acid and extracts it with ether.

If the "albumoses" (Bence-Jones body) are present, Heller's test will give at the line of contact a very heavy ring which disappears on warming and reappears on cooling.

The bile acids will give a precipitate if present in concentrated urines.

If the urine is rich in urea Heller's test may produce at the line of contact a solid crust of urea nitrate which is so solid and so definitely crystalline that it should never deceive one.

Hammarsten recommends that as a routine all urines to be examined by this test be diluted to a specific gravity of 1.005 since then all of the above disturbing bodies will be excluded except albumose and nucleo-albumin.

This test for albumin should always be confirmed by one of the other tests. Many workers recommend that this be the one used first.

POTASSIUM FERROCYANIDE AND ACETIC ACID TEST.—The urine is made quite acid with a few drops of acetic acid and then 5% K_4FeCN_6 is added drop by drop. The presence of albumin will be indicated by a cloud or flaky precipitate. When this ceases to increase no more of the reagent should be added. In the hands of an expert this test is more accurate than is Heller's. Success in its use depends on the proportions of the reagents used and on the amount of salts present in the urine. The test is particularly valuable in quantitative work to determine, *e.g.*, whether or not all of the albumin has been removed from a solution.

The albumoses also are precipitated by this test as is also " Nucleo-albumin," but the latter is also by acetic acid alone.

The urine should not be tested while hot nor should any reagent used contain iron (as Kieselguhr) for this would give an abundant precipitate.

TANRET'S TEST.—Tanret's reagent contains 1.35 gms. of $HgCl_2$ dissolved in as little water as possible together with 3.32 gms. of KI. To this solution are then added 50 c.c. of water and finally 20 c.c. of glacial acetic acid. This reagent is added to the urine drop by drop until the cloud of albumin just begins to appear. This test is exceedingly delicate. It indicates also "nucleo-albumin," "peptone" (soluble on warming), alkaloids and the albumoses. We have seen this test used in French clinics with the most satisfactory results.

SPIEGLER'S TEST.—Spiegler's reagent as modified by Jolles contains $HgCl_2$, 10 gms.; succinic acid, 20 gms.; NaCl, 10 gms.; and water 500 c.c. The specific gravity of this fluid should be well above 1.060 in order that it may be used as a contact test.

This is the most delicate test of all for albumin. The urine is first filtered, rendered acid by a few drops of acetic acid to hold the carbonates in solution and to precipitate any nucleo-albumin, which if present should be filtered off (otherwise it would be precipitated by the reagent). The urine is then superimposed on this reagent (see page 212). If albumin is present a very sharp definite ring will appear at the line of contact. It is claimed that this test will be positive for 1 part of albumin in 150,000 to 350,000 of urine. It is positive also for the albumoses, but not for deutero-albumose.

Various other tests have been proposed. For convenience sake some add to a test-tube of urine a piece of solid metaphosphoric acid or of picric acid the size of a pea. A long list of very delicate tests have been recommended, but there is little to recommend then since it is granted that all normal urines contain a little serum albumin. The important thing is that a worker use 2 tests which control each other well, understand the shortcomings of each and be experienced in their use.

The order of delicacy of the tests mentioned above is: Spiegler's, Tanret's, heat and acid; K_4FeCN_6; Heller's, picric acid, etc. This order, given by Huppert, is not accepted by some, who claim that Heller's test properly performed in a wineglass gives more delicate results even than the heat and acid-test. Senator recommends Heller's test, since it shows albumose. He advised against the heat test, since by its use traces of albumin and rather large amounts of albumose are so often lost. We would recommend for routine work the heat and acetic acid-test after the concentrated salt solution has been added to the urine and that this be controlled by Heller's test.

Quantitative Determination of Albumin.—SCHERER'S METHOD MODIFIED BY COHNHEIM.—To a carefully measured amount of urine (about 500 c.c.) is added 1/10 its volume of a saturated solution of sodium chloride and then it is filtered. About 5 c.c. of this urine is then boiled in a test-tube and filtered. The filtrate is tested for albumin with acetic acid and potassium ferrocyanide. If this test is negative for albumin then the acidity of the whole volume of urine is correct and one should proceed at once with the determination. If, however, this test shows a trace of albumin in the filtrate, then 2 or 3 drops of 50% acetic acid (and only 1 if but a trace was present) are added to the whole volume of urine. This is well stirred and then another 5 c.c. are removed and tested as before. The acidity of the whole volume of urine thus repeatedly increased (or decreased if necessary by a drop of strong NaOH solution) and samples tested until 1 is made albumin-free by heat. Two carefully measured quantities (see below) of the urine, 1 as a control, are now heated in beakers, first on a water-bath and then over a free flame, until the precipitate is flocculent and the supernatant fluid clear, and are then filtered through a weighed filter.

The quantity of urine to use in the analysis should be such that the weight of the dried albumin precipitate will lie between 0.1 and 0.3 gms. If below 0.1 gm., the limit of error is too great; if above 0.3 gm. it is practically impossible to dry the precipitate to constant weight.

Urine which contains much albumin should be accurately diluted with salt solution. If the urine is very rich in albumin, the best method is to pour drop by drop a small, accurately measured amount of the urine into a beaker of boiling, half-saturated salt solution.

When coagulation is completed by boiling the urine over the free flame, the urine is filtered through a dried and weighed filter paper and the precipitate washed free from chlorine (a few drops of filtrate are tested at frequent intervals with $AGNO_3$ solution) with hot water, then with alcohol and finally with ether. The paper containing the precipitate is then placed in a weighed glass (with accurately fitting cover) and dried in an oven, the temperature of which is held at 110° C. The glass in the oven should rest on a sheet of asbestos and the bulb of the thermometer of the oven should hang at the level of the glass. At intervals of about one hour the glass is removed from the oven with a pair of tongs and cooled in a desiccator. The cover is then inserted tightly, and the glass weighed. This is repeated until constant weight is reached. Even with most careful work the controls may differ by even 1%.

SALKOWSKI [101] advised that if there is an unusual amount in the urine a small, accurately measured volume of the specimen be mixed with from 10 to 20 volumes of 95% alcohol and this brought to the boiling point on the water-bath. It is then cooled, the supernatant fluid decanted, the precipitate washed with hot water, filtered through a weighed filter, washed as above, then placed in a weighed platinum crucible and brought in an oven to a constant weight. It is finally burned and the weight of the ash determined and subtracted from that of the precipitate.

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[101] Berl. klin. Wchschr., March 3, 1902.

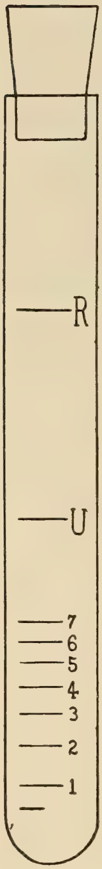

Fig. 35.—Esbach's albuminometer.

Esbach's Tubes.—An Esbach tube (see Fig. 35) is filled to the mark *U* with urine and to the mark *R* with the reagent. The tube is corked, reversed slowly 12 times and then left standing in a tube rack at constant temperature for just 24 hours. At the end of that time the height of the precipitate is noted. The figures on the scale indicate the number of grams of albumin per 1 liter of urine.

Esbach's reagent (picric acid, 10 gms.; citric acid, 20 gms.; water, sufficient to make 1 liter) has not given satisfactory results and has been replaced by Tsuchiya's reagent.

Phosphotungstic acid,	1.5 gm.
Concentrated HCl,	5 c.c.
Ethyl alcohol, q.s. ad	100 c.c.

This method is quite accurate enough for clinical work[102] provided Tsuchiya's reagent be used.

An approximate estimation of the amount of albumin present can be based on Heller's test, made in a "Collamore wineglass" half filled with urine, then underlaid with approximately ⅓ its volume of nitric acid. By "slightest possible trace" is meant the smallest amount of precipitate which can be detected as a hazy ring under most favorable conditions (black background, etc.); "very slight trace" means slightly more; a "slight trace" can be seen without a background and also from above, although the bottom of the glass is distinctly seen; a "large trace" (about 0.1%) is a ring clearly seen but not flocculent, quite dense but not opaque when seen from above; the bottom of the glass cannot be seen through the ring made by 0.15% although a faint ray of light is transmitted; 0.25% of albumin gives a zone quite flocculent from the side and quite opaque from above; 0.5% and above give a ring which is very dense and flocculent. Above this one cannot go by this method. The width of the ring is not so important (condensed from Ogden, "Clinical Examination of the Urine"). This method determines at the same time the "nucleo-albumin" and resinous acids present.

Centrifuge Method.—Purdy recommended the use of graduated centrifuge tubes in which are mixed 10 c.c. of filtered urine, 3.5 c.c. of 10% K_4FeCN_6 and 1.5 c.c. of acetic acid. The urine is then centrifugalized at a uniform speed of 1500 revolutions per minute in a centrifuge, the arm of which is of such length that the distance from the center of rotation to the tip of the tube is 7¾ inches. Each tube is centrifugalized 3 times, 5 minutes each time; ⅒ c.c. volume of precipitate indicates $\frac{1}{60}\%$ by weight of albumin. He gave a table with the equivalents of the readings. This test, satisfactory as it may seem, has not given very good results in our hands, although better than has the Esbach tube. It is an interesting fact that 2 of the makers of the "Purdy centrifuge" were unable to supply us with an arm which conformed to his specifications as regards length (hence they had to be made to order) or with graduated tubes with the sharp point as he represents them. We have found it is no easy matter to keep a centrifuge running uniformly at the rate specified unless one carefully watches the taxometer and yet the exact time and speed of rotation are of great importance.

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[102] See Mattice, Arch. Int. Med., March, 1910, vol. v, p. 313.

Roberts and Stolnikow's Method.—This method is based on the observation that if with Heller's test a ring appears in from 2½ to 3 minutes after the test is made there is an albuminuria of 0.003%. Different dilutions of the urine are therefore tested until there is one obtained in which the ring appears in 3 minutes. The test should be performed very carefully. The sides of the tube should not be wet with the nitric acid and the urine should be added slowly from a pipet.

It is often necessary to *remove all albumin from a urine* before undertaking other quantitative work. Usually it is sufficient to add acetic acid and to boil until the filtrate is clear (see page 215), and then restore the urine to its original volume. Hofmeister's method is more accurate. He adds to the urine an excess (10 c.c.) of a 40% solution of sodium acetate and concentrated Fe_2Cl_6 until the specimen is of a red color. The urine is then neutralized or made very faintly acid and boiled. The precipitate of basic ferric acetate which forms will carry down with it all of the albumin and leave an albumin- and iron-free solution which filters beautifully. This method cannot be used if glucose is present since then some ferric oxide will remain in solution.

PROTEIDS PRESENT IN THE URINE

By albuminuria is meant the presence in the urine of a coaguable protein. A true albuminuria is one which is due to disturbance of the cortex of the kidney (for false albuminuria, see page 224). The proteins usually present are serum albumin, "serum globulin," and the so-called "nucleo-albumin."

The *albumin quotient* is obtained by dividing the amount of serum albumin by the amount of "serum globulin" present (Hoffman). This quotient varies considerably in different cases, and in the same cases at different times. In some cases serum albumin alone has been found. This was true of 1 case of cancer of the stomach, and during limited periods of certain cases of nephritis. Globulin alone was found in 1 case of acute nephritis, in 1 case during the puerperium and in 1 case of leukemia. (For "nucleo-albumin," see page 219.)

Serum albumin is present in normal urine in amounts reaching even from 22 to 78 mgms. per 1 liter (Mörner). Serum albumin is soluble in water and is coagulated by heat, if in acid solution, at a temperature varying from 56° to 81° C., depending on the amount of urea and of salts (especially of phosphates) present and lastly on its own concentration. It is coagulated by alcohol, which coagulum if produced by absolute alcohol is soluble in water unless it has been in contact with the alcohol for a long time. The coagulum produced by weaker alcohol is more insoluble than that produced by stronger. The solubility of this coagulum should always be borne in mind when working quantitatively with albumin. Serum albumin is levorotatory, $[\alpha]D = -62.6°$. Serum albumin unites with an alkali to produce a soluble body which, when combined with a base, forms an albuminate much less soluble in water than is albumin itself and which may explain the spontaneous precipitate of albumin in some concentrated urines.

The acid albumin produced by mineral acids is quite insoluble except in large excess of the acid while that produced by acetic acid is soluble in a very slight excess of the acid.

Serum globulin is a term under which are included several quite different proteins, among them pseudoglobulin, euglobulin, and fibrinoglobulin (Hofmeister), all of which exist in the blood-plasma and the reactions of which are rather different. Euglobulin and fibrinoglobulin (fibrinogen) are probably always present in the normal urine. It is they, slightly increased, which explain the mildest forms of albuminuria (the so-called physiological cases), while in severer cases serum albumin also is increased.

These globulins may be separated by fractional precipitation by a saturated $(NH_4)_2SO_4$ solution. The limits of precipitation expressed in the number of cubic centimeters of the ammonium sulphate solution in the 10 c.c. mixture are: pseudoglobulin 3.4 to 4.6; euglobulin 2.8 and 3.3; fibrinoglobulin, 2.2 to 2.9.

PSEUDOGLOBULIN is not precipitated by acetic acid alone.

EUGLOBULIN occurs in almost all exudates and transudates and in many urines, perhaps in all. It can sometimes be precipitated by acetic acid in the undiluted urine, but usually one must dilute this with 2 or 3 volumes of water. The acetic acid must be carefully added since the precipitate is easily soluble in excess.

SERUM GLOBULIN, including under this term all the above globulins, is present in the urine in cases of albuminuria in amounts varying from 8 to 60% of the total proteid present; very rarely a trace only is present. In the blood its ration to serum albumin is as 1 : 1.5. Its greater relative ratio over the albumin in the urine cannot always be explained by its greater diffusibility, since euglobulin, which is constantly present, is little diffusible. The variations in the albumin quotient (see page 217) in nephritis are due to variations in the amount of globulin. Oswald considered that in the mildest form of albuminuria euglobulin alone is present in pathological amounts and that this is precipitated by acetic acid in the cold. This body is excreted in largest amounts in parenchymatous renal lesions. As a case of nephritis improves the relative amount of globulin in the urine diminishes, to increase with each acute exacerbation. In cases of contracted kidney and in nephritis with chronic passive nephritis it may be very low. It is fairly low in the albuminuria of pneumonia, but high in that of typhoid fever.

The globulins are insoluble in distilled water. In the urine they are held in solution by the salts present. If, therefore, to a beaker of distilled water a drop or so of urine containing much globulin be added, a distinct cloud will appear. They may be detected also by diluting the urine till its specific gravity is about 1.002 and then adding 1 drop of acetic acid.

To *isolate the globulins* the phosphates are removed by rendering the urine alkaline with ammonia and filtering. An equal volume of cool saturated ammonium sulphate is added to this filtrate, which will precipitate the globulins perfectly in neutral solution. The mixture is allowed to stand 1 hour and filtered. The precipitate which contains also albumose and " nucleo-albumin " is washed with half-saturated ammonium sulphate until the filtrate is albumin-free. Ammonium urate also may be precipitated in time and is to be avoided by working fairly rapidly. Serum albumin will not be precipitated until the point of total saturation with the sulphate is reached. The precipitate on the filter is now dissolved in a little water and heated on a water-bath which will coagulate the globulin, fibrinogen and albumose. This is filtered and the precipitate washed with water and digested on a water-bath with 1% soda. This solution is then filtered and neutralized carefully with acetic acid. The precipitate which now falls consists of globulin and fibrinogen, but not albumose.

To determine globulin quantitatively the filtered urine is first rendered neutral with ammonia and is then mixed with an equal volume of saturated ammonium sulphate solution. The mixture, well stirred, is allowed to stand for several hours. It is then filtered through a dried and weighed filter and the precipitate washed with half-saturated ammonium sulphate until chlorine-free. This filtration is a tedious process. The funnel containing the precipitate on the paper is then placed in a thermostat and dried for ½ hour at 110° C. The ammonium sulphate is now washed out with hot water, the precipitate dried with alcohol, then with ether and dried at 110° C. to constant weight (see page 215). In this determination also the amount of urine used should be such that the weight of the protein precipitate will not exceed 0.3 gm.

EUGLOBULIN, NUCLEO-ALBUMIN, MUCIN, MÖRNER'S BODY.—If one adds to cold urine, and especially if this be well diluted, a few drops of dilute acetic acid, there often appears an opalescence or even a true precipitate, which is difficultly soluble in an excess of the acid. When using Heller's test also one often sees a ring, not at the line of separation, but from 0.5 to 1 cm. above it. If serum albumin is present one may see both rings, and indeed the upper, the "nucleo-albumin" ring, is best seen in the urine of nephritis. The resinous acids and the urates should be excluded (see page 213). This proteid, commonly called nucleo-albumin, which explains these 2 phenomena, coagulates at about 56° C. It could be demonstrated in probably every normal urine if the salts be first removed by dialysis.

It was this body which led to the belief that at least one true proteid is a constituent of normal urine. Two other and contrary views have been held, one that this body is mucus, the other that it is true nucleo-albumin. If it were either of these, the condition would not be a true albuminuria. At present most believe that this substance is globulin or a compound of serum albumin and that a true albuminuria is normally present.

A definite precipitate (indicating a definite increase of the protein) on the addition of acetic acid to the cold urine is seen in many conditions. Excluding the vesical cases, in which the substance is probably mucus, it is increased in the new-born; in adults after severe exercise; in nephritis; in various acute diseases, especially those affecting the kidneys; in fevers, especially pneumonia and typhoid (also erysipelas, pleurisy, relapsing fever, meningitis, etc). Its increase in leukemia, reported first by Fr. Müller, gave rise to the idea that it was derived from the nuclei of the leucocytes.

Obermayer found it in 32 cases of jaundice in amounts varying with the intensity of the jaundice; in scarlet fever in small amounts, in diphtheria in the greatest amounts of all; after poisons affecting the kidneys (pyrogallic acid, corrosive sublimate, etc.); in acute yellow atrophy and after compression of the thorax.

In true nephritis the increase of this proteid may precede the true albuminuria and also may succeed it as the case improves. Madson considers it evidence of the earliest possible irritation of the kidneys. It may persist during the intermissions of an albuminuria. Euglobulin and fibrinogen are said to be the chief proteids in the urine in amyloid disease.

In orthostatic albuminuria this may be the only proteid in the urine, or it may be accompanied by serum albumin and pseudoglobulin. In febrile albuminuria this may exceed in amount the serum albumin. It is present in traces in chronic interstitial nephritis. When the blood-supply of the kidney of animals is partly cut off, this body may be excreted in abundance, sometimes alone and sometimes with albumin. The same is true in partial suffocation of the organism.

Pure mucus is present in the normal urine in traces (4.5 gms. in 260 liters) and in 2 forms, an insoluble form which gives the nubecula and a soluble portion precipitated by acetic acid, which constitutes but a very small fraction of the whole. One would expect to find some mucus in the urine since the urinary passages are lined with mucous membrane the secretion of which will be washed off by the urine. This mucus is greatly increased in catarrhal conditions of the urinary tract. It is soluble in ammonia, is precipitated by acetic acid and is soluble in excess of this acid. From it a reducing body may be split off. This mucus does not contain nuclein nor chondroitin and the precipitate with acetic acid lacks the slimy character of true mucus thus treated. For this reason it has been called a " mucoid " body. It resembles the ovomucoid of the hen's egg (Mörner). In the urine of a case of prostatitis we were able to obtain by precipitation with acetic acid 0.066 gm. of this substance per 100 c.c. of urine.

To determine the mucus quantitatively the urine is precipitated carefully with acetic acid and repeatedly filtered through a weighed filter till the filtrate is clear. The precipitate is then washed with cold water acidulated with acetic acid, dried and weighed. Another method, much more rapid than the preceding but giving slightly lower results, is the following: A small amount (0.5 gm.) of infusorial earth dried at 110° C. to constant weight, is mixed with the urine after the mucus has been precipitated. Then the urine is filtered, the precipitate dried and weighed. Then the weight of paper and of the Kieselguhr are subtracted.

There are on record interesting and rare cases of true mucinuria which are analogous to mucous colitis and to fibrinous bronchitis. In these cases mucus casts 1 to 10 cm. long and 3 to 4 mm. thick may be voided with the urine. Such was v. Jaksch's case of "ureteritis membranacea" in which a spiral cast of the ureter consisting of mucus and fibrin was voided; Frank's case which he named " pyelitis productiva " passed a cast of the pelvis and upper ureter. Four cases only of this condition are on record. In these cases the symptoms of the passage of the casts resembled those of renal colic.

Nucleo-albumin.—From a study of the urine of jaundiced patients Obermayer decided that the proteid which in that condition is precipitated by acetic acid is true nucleo-albumin and assumes that all precipitates by acetic acid in the cold are the same. True nucleo-albumin may be present in the urine, but this is not the body which usually goes under that name and its presence is never normal. It is said that its source is the broken-down epithelial cells of urinary tubules, as in acute nephritis, in which condition this proteid is most often present and in greatest amounts, after the ingestion of poisons which affect the kidneys, in disturbances of the renal circulation and finally jaundice, in which case its source is the bile itself. Nucleo-albumin is said by some to be a constant constituent of the blood, and it is possible that a certain amount of this may reach the urine. True nucleo-albumin, it is said, is found in the urine in cases of catarrhal inflammation of the urinary tract with desquamation of the superficial cells of the mucosa, as in cystitis or pyelitis. In the case of women the genital tract is to be excluded as the source.

It is apparent from the above list that nucleo-albuminuria is said to occur in conditions in which one might expect it, and yet one does not find it where nucleo-albumin should be present in the urine in largest amounts, as in urines containing abundant pus and epithelial cells. Here it is often hard to get any precipitate at all on adding acetic acid.

It is very clear to one reading reports of cases of so-called nucleo-albuminuria that seldom has the crucial test, the proof that the substance in question contains phosphorus, been applied. (It also would of course be difficult to prove this, since it would be very hard to exclude a contamination with the inorganic phosphorus of the urine.)

Again, the " salting out " points with ammonium sulphate of the so-called nucleo-albumin of the urine do not quite agree with those of true nucleo-albumin obtained by the breaking down of tissue, which are[103] minimal 0.1 to 0.8, maximal, 1.6 to 2.2.

To prove that a body is nucleo-albumin it would be necessary to show that it is insoluble in acetic acid, is precipitated by $MgSO_4$, that when boiled with dilute mineral acids it gives off no reducing substance and that peptic digestion gives nuclein and phosphorus. The last 2 tests it is almost impossible to apply to the urine.

Mörner's Body.—Much light was thrown on the subject of nucleo-albuminuria by Mörner[104] who proved that most of the so-called nucleo-albumin of the urine is a compound of true serum albumin with an albumin-precipitating body which is formed when acetic acid is added to the urine. By dialyzing large amounts of urine, adding 1 to 2 parts per thousand of acetic acid, and then shaking out the residue with chloroform, Mörner obtained a precipitate averaging 41 mgms. (22 to 78 mgms.) per liter of urine which resembled nucleo-albumin. This proved to be a combination of serum albumin and certain other bodies, among them chondroitin-sulphuric acid, which was always found and in the largest amounts, nucleinic acid, which is sometimes present in traces, and taurocholic acid, which is often present in traces but which in the case of jaundiced urine may exceed in amount the other 2. The fact that 3 different compounds of serum albumin may arise and in varying proportions explains the lack of uniformity in the properties of the precipitates which appear when acetic acid is added to the urine. This combination of albumin and these acids probably occurs after the acetic acid is added. If after removing this precipitate a little albumin is added to the urine a second precipitate will appear, amounting to about 54 mgms. per liter, which shows that the albumin-precipitating bodies are in excess of the albumin. The larger the proportion of serum albumin in this combination the more will the compound react like serum albumin, but the greater the relative predominance of these precipitating bodies, the more will it resemble nucleo-albumin.

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[103] Matsumoto, Deutsch. Arch. f. klin. Med., 1903, vol. lxxv, p. 398.
[104] Skand. Arch. f. Phys., vol. vi, p. 332, 1895.

It is this compound of albumin which explains the statements based on common experience, that " a true albuminuria is sometimes preceded by the excretion of a body precipitated by acetic acid "; and that " the excretion of mucus may precede or succeed an albuminuria." It explains also the belief of recent years that this so-called physiological albuminuria was merely a nucleo-albuminuria.

Mörner used the following method of isolation: The salts of a large volume of urine are removed by dialysis and acetic acid then added, 2 c.c. per liter. The precipitate formed is then dissolved in a little water and again precipitated with acetic acid. A little is then heated for a long time on the water-bath with 5% HCl. If sulphuric acid is present but no reducing body can be demonstrated chondroitin-sulphuric acid is probably present; if the reducing body can be demonstrated, but not sulphuric acid, the precipitate was probably mucus. If no sulphuric acid and no reducing body can be demonstrated, the precipitate should then be digested with pepsin and the products examined for organic phosphorus. If this phosphorus is present the nuclein bases should be tested for.

This explanation of Mörner, satisfactory as it would seem and evidently based on very careful work, has received but little confirmation. Stähelin [105] in 1 case of jaundice failed to find any of the " albumin-precipitating bodies " and thought the precipitate on adding acetic acid resembled the globulin, a view held by Fr. Müller in 1885; also in the very heavy acetic acid precipitate of the urine of a case of pneumonia no phosphorus could be detected. Matsumoto found this substance to consist chiefly of fibrinogen and euglobulin (see page 218). Oswald [106] studied this precipitate in the urines of cases of cyclic albuminuria and nephritis, and he also decided it to be euglobulin and a trace of fibrinogen. These proteins occur in the blood, but cannot be demonstrated in that fluid by the addition of acetic acid, since the salt content there is too low.

It is to be noted that most observers have worked with smaller amounts of urine than Mörner; again, that it is not proven that the limits of precipitation with saturated ammonium sulphate are sufficient for the recognition of a protein. In conclusion, it is clear that, however, the present conflict between Mörner and the Hofmeister school may be settled, both agree that there is a constant normal physiological albuminuria.[107]

The NUCLEOHISTON of LILIENFELD is a body arising from the breaking down of leucocytes. It is precipitated by acetic acid and has a high phosphorus content. It has been found in large amounts in the urine of leukemic patients, although its appearance there is not due entirely to the breaking down of white cells.

To demonstrate nucleo-histon in the urine all coagulable albumin is first removed. Then all other proteids are precipitated with alcohol, the precipitate washed in hot alcohol, then dissolved in boiling water, cooled, acidified with HCl, let stand and the uric acid precipitate filtered off. To the filtrate is then added ammonia, the resulting precipitate is collected on a small filter and washed with ammonia till the wash-water gives no biuret reaction. The precipitate is then dissolved in acetic acid and tested for histon. This will give the biuret reaction, is coagulated by heat and this coagulum is soluble in mineral acids.[108]

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[105] Münch. med. Wochenschr., 1902, p. 1413.

[106] Zeitschr. f. d. gesamt. Biochem., Bd. v., 1904.

[107] See also Calco., Zeitschr. f. klin. Med., 1904, vol. li.

Fibrinuria.—Fibrinogen or fibrinoglobulin is found in the urine rarely in demonstrable amounts. Its chemical reactions are those of globulin but its presence is indicated by the appearance of spontaneous coagulation if the urine is left standing. Excluding those cases in which there is considerable blood in the urine, fibrinuria is rare. It occurs in chyluria and rarely in nephritis. In some of these cases the urine clots at once after it is voided. The clot is sometimes firm and in other cases gelatinous. We have seen but 1 clear case, a woman admitted during the last hours of her life with what was evidently subacute parenchymatous nephritis. Only about 5 c.c. of urine could be obtained. This was cloudy, yellow in color and after standing for a few minutes clotted to a solid coagulum. In other cases reported the urine clots before it is voided and casts of the pelvis of the kidney or bladder are passed. In severe inflammation of the bladder, ureter, or pelvis of the kidney, such clots sometimes form. Why, is not known, since inflammatory exudates as a rule do not coagulate. In any decomposing alkaline urine, masses which resemble fibrin casts consisting of pus, mucus and bacteria may be voided or may even plug the urinary passages.

Albuminuria.—Albuminuria or the presence of a coagulable protein in the urine, albumin, serum albumin, serum globulin, etc. (see page 217), may be due to conditions in the renal cortex (true, or cortical albuminuria) or to conditions below the cortex (" false " or, better, sub-cortical albuminuria). In cases of sub-cortical albuminuria the urine as secreted by the kidney cortex is normal and receives the albumin lower in the urinary passages, either as an inflammatory exudate, lymph, blood, or chyle. By albuminuria in the following paragraphs is meant that due to some disturbance of the renal epithelium, especially that of the glomeruli.

Albuminuria Without Definite Renal Lesion.—Physiological albuminuria, or the constant presence of a proteid in normal urines has been mentioned on page 223. Posner first, in 1884, claimed the presence of serum albumin in all normal urines, but this was at once doubted, since the tests seemed to indicate that the proteid present was mucin or a nucleo-albumin. More recent work, however (see page 218), seems to have established beyond doubt the presence of a small amount of serum albumin, or, according to others of euglobulin and directly from the blood, in practically all normal urines. If we use Speigler's reagent it would be hard indeed to find many whose urine is really albumin-free.

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[108] Kolisch and Purion, Zeitschr. f. klin. Med., 1896, Bd. 29, p. 374.

The difference between physiological and pathological albuminurias is quantitative not qualitative and the term "albuminuria" now implies that serum albumin is present in the urine in such quantities that it can be detected by the not very sensitive tests accepted as standard, *e.g.*, the heat and acetic acid test is standard in our clinic. Hofmeister's standard was that Heller's test should show no ring in 3 minutes.

The question of albuminuria is, therefore, similar to that of glycosuria; very small amounts of both bodies are found in normal urines but are disregarded unless present in amounts sufficient to give the test accepted as standard. This line is, however, an artificial one and not very convincing to the medical students who are sorely tempted to try the more delicate tests on their own urine and are made unhappy as a consequence.

The problem of physiological albuminuria now little interests the clinician. By albuminuria he means albumin in pathological amounts and the older discussion of physiological albuminuria must now be continued under the title "functional albuminuria." The question now is: May the normal person under practically normal conditions pass a urine containing enough serum albumin to give the heat and acetic acid test or Heller's test within 3 minutes?

FUNCTIONAL ALBUMINURIA.—The term "functional" albuminuria Pavy used merely as a contrast term to "structural" albuminuria in which latter case the albuminuria depended on demonstrable anatomical changes in the kidney.

Senator defined an albuminuria as "functional" (he said "physiological") if it occurs in young men, is transitory, is slight in grade, if the further history of that person is negative, if the urine is otherwise normal and if its occurrence follows an unusual cause, such as severe muscular work by one not used to it; exposure to cold, nervous stress, or after unusually large proteid meals. According to Senator the cause need be an unusual one solely for that person and at that time.

This form of albuminuria was first noticed among soldiers. Leube stated that 59% of the raw German recruits showed a temporary albuminuria after a forced march, but not later after they had been strengthened by training. Macfarlan [109] found albuminuria in practically every foot-ball player for hours after a game, while in the urine of 19 he found granular casts as well and in that of 6 also blood-casts and red-blood-cells. Müller [110] found albuminuria in the urine of 11 bicycle riders after a race and in several of these he found also casts of all descriptions and renal epithelium. Their urine was normal on the following day. Barach [111] found in the urine of each of 19 Marathon runners albumin, casts, and, in nearly all cases, blood. One week later the urine of 4 of these men still contained casts and albumin and that of 2 casts alone. In other words, an albuminuria may be expected in any athlete no matter how good his condition, if only his exertion be strenuous enough and especially if the exertion involves principally the leg and thigh muscles. Normal man in every sphere of life, even the trained athletes, have certain limitations and the question arises, having overstepped these, can the albuminuria which results be termed "functional" even though the persons involved would be considered almost perfect physically (trained athletes, *et al.*)?

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[109] New York Med. Record, 1894, vol. xvi, p. 769.

[110] Münch. med. Wochenschr., 1896, No. 48.

[111] Arch. of int. Med., Apr., 1910, vol. v, p. 382.

In this same group of functional cases Senator includes the albuminurias which follow violent emotions or an unusually heavy proteid meal (alimentary albuminuria). Among soldiers, normal men under uniform conditions, Rapp found that 10.7% showed albuminuria after their mid-day meal. Experiments show that the ingestion of excessive amounts of certain proteids will cause an albuminuria in some apparently normal persons. This is especially true if egg albumin be used. Such an albuminuria begins in about 2 hours after the meal and lasts about 4 hours.[112] Formerly it was claimed that the proteid ingested can itself be detected in the urine but the identification of proteins by the specific precipitin method has not proven very satisfactory. Some raw egg albumin may be excreted as such but even then the most of the protein present will be serum albumin, showing that the foreign proteid in the blood temporarily at least injured the kidneys. Certainly we know that if the kidneys are inflammed any indiscretion in the diet may aggravate the condition. An alimentary albuminuria is claimed for the new-born if fed on cow's milk and explained on the ground that their intestinal mucosa has not yet developed its normal impermeability to foreign proteids.

Prolonged cold baths will cause an albuminuria. Rem Picci observed 115 baths taken by 35 healthy men and found that a bath of 3 minutes at 12° to 13° C., or 15 minutes at 15° to 20° C., caused quite uniformly a slight transitory albuminuria, minimal in amounts, never lasting over 24 hours, together with casts and generally with diuresis with increased urea and chloride output.

Mental over-exertion is said in certain cases to cause albuminuria.

Albumin is more likely to appear in the urine should several of these above-mentioned factors occur simultaneously. The intermittent nature of the albuminuria is no proof that it is functional since a truly pathological albuminuria may intermit for long periods of time and in a mild case, or one during convalescence, the albuminuria may return for but a few hours after 1 of the above causes. Certainly the amount is not important although Senator considers that if it exceeds 0.4 to 0.5 gm. per liter it cannot be called functional (he said "physiological").

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[112] See Ascoli, Münch. med. Wochenschr., 1902, No. 10, and Inouye, Deutsches Arch. f. klin. Med., 1902, Bd. 75; and on the opposite side of the question Umber, Berl. klin. Wochenschr., July 14, 1902; for the chemical side, Sollman and Brown, Jour. of Exp. Med., March 17, 1902.

Another example of the so-called "physiological albuminuria" is the albuminuria of the new-born. The urine of many infants during the first 8 or 10 days after birth contains a slight amount of albumin, hyaline casts and epithelial cells. Ribbert gave as an explanation that the kidneys at birth often are not quite finished; that is, that there is still to occur a desquamation of the epithelium of the capsules of the glomeruli. The urine found in the bladders of stillborn infants often contains albumin and casts, but this may have another explanation.

THE ALBUMINURIA OF WOMEN DURING PREGNANCY AND IN LABOR.—An albuminuria may be considered as truly physiological which follows the confinement. It usually disappears at once. Little, [113] as the result of very careful work, concludes that albumin may be demonstrated in the catheterized urine of about one-half of all pregnant women, of primiparæ as often as of multiparæ, although casts are found more often in the urine of the latter. During uncomplicated labor a still greater number, and especially of the primiparæ, show albuminuria. This may be due to the muscular work and the increased blood-pressure which childbirth involves.

"ALBUMINURIA OF ADOLESCENCE" (Gull), "of puberty"; "accidental albuminuria"; "essential albuminuria"; "physiological albuminuria"; "Pavy's disease"; "cyclic albuminuria of the apparently healthy"; "postural," "orthotic," "orthostatic" and "intermittent albuminuria." From this list of names one may make out the essential features of the various cases. Posner proposes the quite satisfactory term, "essential albuminuria," for albuminuria is the one and only symptom common to all. These cases are of far greater importance clinically than those discussed under the title "functional" albuminuria. They form a large group of persons who enjoy reasonably good health, but whose urine either constantly or intermittently contains a trace of albumin. This condition is discovered by Army and Navy medical inspectors, by the examiners for insurance companies and by the doctors to whom neurasthenics apply for treatment. Insurance men say that 5% of all the "normal" persons examined while the temperature is above 90° or below 0° F. show albuminuria; at other times, only about 2%.

Of the group as a whole it may be said that these individuals are for the most part youths or young adults who are not robust. Some are anemic and many complain of symptoms referable to instability of the vasomotor system. Many come of neurotic families while more have had those infectious diseases which often are followed by nephritis; such as tonsillitis, scarlet fever, diphtheria, etc., although they have no signs or symptoms of kidney trouble other than an albuminuria which may be continuous, or if intermittent appears in response to some ordinary acts of everyday life and not to unusual causes. The tendency now is to eliminate from this group all in whom latent infection can be demonstrated, whether of the tonsils, teeth, nasal sinuses, gall-bladder, appendix, tubes or prostate (some would add the bronchial tree and the colon wall) on the ground that these patients have an actual subacute nephritis; also, all those who are anemic (and so probably harbor a latent infection) or have had recently an infectious disease. Essential albuminuria is sometimes a family disease present in 2 or even 3 children of one family. In this group some include the albuminuria of masturbators and that which follows sexual excitement. In these cases the albumin often is present only in the morning specimen. Some (Sir Andrew Clark and others) say that this proteid is from a secretion of the urethra or accessory sexual glands.

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[113] Amer. Jour. of Obstet., vol. 1, No. 3, 1904.

A diagnosis of essential, etc., albuminurias is possible only after a long, careful study of the individual case, including his past history, a careful physical examination especially of the circulation and eyes, an accurate study of the urine with especial reference to its specific gravity, amount, sediment, etc., all have failed to reveal any other evidence of renal disease and even then an autopsy may reveal a true nephritis. The fact that the albuminuria is intermittent or is orthostatic, or is, or is not, accomplished by a cylindruria, does not help to exclude a latent acute nephritis during convalescence.

Leube taught that the cases of albuminurias of adolescence formed a separate, distinct group. In them the albuminuria is present only between the ages of 14 and 18 years and then disappears. It is attributed to an insufficiency of the kidneys relative to the growing organism, associated with instability of the vasomotor centers. In this group are found most, but not all, of the cyclic or postural cases. In them the element of heredity would seem to be important. The cases reported by Lommel [114] would fall under this title. He reported that of 587 factory workers from 14 to 18 years old, 18.9% showed slight albuminuria once or many times and either no sediment, or at the most a few hyaline casts and fatty epithelial cells in the centrifugalized specimen; of 130 patients from the same class but over 25 years old, only 1 showed albuminuria. Functional cardiac and vascular disturbances were common among these workers. Posner emphasized sexual excesses at puberty as a common cause of this form of albuminuria. Sutherland [115] noted a definite relationship between albuminuria and the movable kidneys present in ⅓ of his cases.

Cyclic albuminuria is the most interesting of all so-called physiological albuminurias. In these cases one can demonstrate a remarkable daily cycle; the albumin is absent at night and when the patient lies flat on his back but appears whenever he stands up. The terms "orthostatic" or "postural" albuminuria therefore are much preferred by some for this group although by usage the term cyclic albuminuria implies one not due to nephritis, while the best examples of orthostatic albuminuria are seen in cases of mild nephritis.

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[114] Deutsches Arch. f. klin. Med., 1903, Bd. 78, p. 541.
[115] Am. Jour. of the Med. Sci., 1903, vol. cxxvi.

The cyclic cases may be subdivided into 3 groups: those associated with vasomotor phenomena (the neuropathic element predominating), those with abnormal renal circulation, as in congenital floating kidney, and the hereditary form. Mix also [116] recognized an intermittent and a continuous type of cyclic albuminuria. In these intermittent cases the albumin as a rule appears after rising, reaches a maximum at noon or from 4 to 6 P.M., then declines and disappears from 8 to 10 P.M. If the patient changes his habits the cycle will change also. In many cases the albuminuria bears little or no relation to meals. The cycle affects not only the albuminuria but also the water output (which decreases as the albumin appears). As a rule one can note the following sequence: first, an increase in the pigments, then the appearance of albumin, then an increased output of uric acid and lastly an increase of urea (Teissier). While casts are rare, yet careful search will, as a rule, discover a few. This albuminuria may even be diminished by exercise and fatigue. The cycle in the continuous form continues for years and if it ceases does not return. These cases practically never develop into Bright's disease. The adults of this group are neurasthenics with vasomotor instability and 37.5% of the children have a congenital movable kidney. Armstrong [117] found this form of albuminuria in 12 to 15% of over 3000 school boys. It is seen more in summer than in winter; heredity is often present; it is often associated with depression of spirits and fainting spells, especially while the boy is idle, not when occupied; the boy is apathetic, his heart is subject to intermittent attacks of dilatation and palpitation; it lasts only during puberty.

It is an open question whether cyclic albuminuria should be considered as physiological or pathological. When this discussion began nephritis, no matter how slight, was thought of as a disease which, once begun, was likely to continue for years or even for life. With better knowledge of the importance of latent infections in the production of nephritis and the excellent prognosis when these cases are properly treated, one reason for the use of the term "cyclic albuminuria," has in large measure disappeared. Most transitory albuminurias certainly are pathological, *e.g.*, those of fevers. Posner's case was well after 17 years. Senator [118] insisted that the majority of these cases have no nephritis. Krehl, who followed several cases over a long period of time, considered this condition harmless and not a form of mild nephritis. Broadbent [119] has never known a true case of this form to develop actual rental disease. In all the above cases the amount of albumin is small, the amount of urine normal and its specific gravity normal. A few hyaline casts may or may not be present. There are no cardiovascular changes. The immediate cause is much in dispute. Possibly the most reasonable explanation will be one which associates the urinary findings with changes in the renal circulation which follow changes in posture. Edel found in 3 very interesting cases that the albumin-free intervals (in the afternoon as a rule) were also periods of diuresis and that in some degree the amount of albumin varied inversely to that of the urine. Erlanger and Hooker [120] found that the amount of albumin varied inversely as the pulse-pressure. Many of these cases later recover. The last and best review of this subject is that of Hooker.[121]

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[116] Am. Jour. of Med. Sc., 1904, vol. cxxviii, p. 307.
[117] Brit. Med. Jour., 1904.
[118] Deut. Arch. f. klin. Med., December 8, 1904.
[119] Brit. Med. Jour., 1904.

The diagnosis of cyclic albuminuria often can be made only after years of observation, since many patients who first are included in this group later prove to be cases of true Bright's disease.

The hypostatic albuminuria of splenic origin seen in some patients with enlarged spleens while recumbent and absent while they are erect, is, Rolleston thinks, the opposite of cyclic albuminuria. Since it is not seen, however, in all patients with enlarged spleens nor in those whose spleens are largest, some other causal factor is necessary. Pressure on the left renal vein may explain some of the cases. This may resemble the albuminuria in chronic passive congestion of mitral disease.

Albuminuria Minima (Lecorché and Talamon).—Under the heading "albuminuria minima" are included cases whose urine constantly contains a trace of albumin, but almost never over 0.5 gm. per liter. The output varies very little with the position of the patient, the time of day, diet, etc., although for each patient there may be some individual factor which increases the output. Some of these cases are quite certainly convalescent from a latent subacute nephritis. The prognosis is uncertain and must be guarded, for some later are cases of a true nephritis. Others remain the same for years with no further symptoms. Under this group the French put the post-infectious cases, albuminurie résiduale, albuminurie paracellaires (or insular nephritis), albuminurie cicatricielle (due to imperfect healing, leaving a "scar"); also the albuminuria of adolescence, the hereditary form, albuminurie phosphaturique, and the albuminurie prégoutteuse.

Intermittent albuminurias are those which persist for periods lasting weeks, months or years and then cease for longer or shorter periods. This term does not include the cyclic or postural albuminurias in which the albumin-free periods last for hours only. They usually are cases of insidious nephritis which have followed some preceding acute infectious disease. In other cases the albuminuria, with casts often, is due to and is present only during periods of broken cardiac compensation.

The intermittent heredity form of albuminuria includes, according to some, many patients who formerly showed the albuminuria of adolescence, but who now in adult life are albumin-free except in response to fairly adequate causes.[122] In some cases the parents of the patients had had albuminuria during youth, while in others a neurotic family history is the only suggestive feature.

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[120] Johns Hopkins Hosp. Rep., vol. xii, 1904.
[121] Arch. of Inter. Med., 1910, vol. v, p. 491

Traumatic Albuminuria.—Transitory albuminuria may follow injury to the brain, as apoplexy; injuries which crush the kidneys, in which cases the albuminuria and cylindruria may continue for a long time without the appearance of other signs of nephritis, although this may explain some cases of so-called benign latent contracted kidneys (Stern, Curschmann); bimanual palpation of the kidney, even that of an ordinary physical examination (Menge [123] could in 15 of 21 cases cause by bimanual palpation a transitory albuminuria lasting usually from 1 to 24 hours and in some cases also a slight hematuria); and finally anything obstructing renal venous flow, as in movable kidney during Dietl's crises.

Febrile Albuminuria.—During any acute fever, but especially pneumonia, typhoid, malaria, acute articular rheumatism, grippe and acute tonsillitis, there may be a slight albuminuria which begins with the fever and disappears with it. The renal lesion in such cases is thought to be the cloudy renal epithelium, the faintest grade of inflammation (Leyden). The amount of albumin in the urine of these patients usually is small, but sometimes is great. Hyaline and epithelial casts are often found but no other formed elements suggesting inflammation. These cases differ from true cases of nephritis only in degree.

Under the term hematogenous albuminuria is included a very large and confusing group of non-febrile cases which show at autopsy no renal lesions, except, perhaps, slight parenchymatous changes. In this group occur some of the cases of purpura, scurvy, chronic lead or mercury poisoning, lues, leukemia, cachexia, severe anemia, cholemia, hyperglycemia and ether and chloroform narcosis.

"Hematogenous albuminuria," accurately speaking, would indicate one due to the elimination by normal kidneys of some proteid in the blood which cannot be used. Cases in which there is a possibility that the renal epithelium has been injured, as by the proteid itself or some poison as lead, mercury, etc., should be excluded. It is true that foreign proteids, *e.g.*, albumoses, egg albumin, peptone, casein, free hemoglobin, etc., if introduced into the serum will appear in the urine and formerly this was the explanation (an unfit proteid) of all forms of albuminuria, but the chances are that all are due in part at least to some disturbance of the renal cells themselves, for they certainly are exceedingly sensitive to changes in their nutrition. Indeed a true hematogenous albuminuria has not yet been proven.

The Nervous Form.—Patients with epilepsy, apoplexy, tetanus, exophthalmic goiter, injuries to the head, delirium tremens, various psychoses, even neurasthenia and migraine, often have a slight transitory albuminuria. In some cases a few casts also appear. In other very interesting cases casts and no albumin are found. We followed for several weeks the urine of such a case, a boy 14 years old with hysterical attacks followed by cylindruria. A very transitory early albuminuria could not be excluded, but not one of the specimens examined contained albumin.

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[122] Dieulafoy, Loude, Arch. gén. de méd., n.s., ii, 3, p. 257, 1899.
[123] Münch. med. Wochenschr., June 5, 1900.

Closure of the ureter, retention of urine in the bladder, compression of the thorax, have been accompanied by albuminuria; digestive disturbances, as obstruction of the bowel (a reflex cause being assumed as in cases of strangulation of the bowel or omentum[124]), acute diarrhea, constipation and liver disease are sometimes given as causes. In ⅔ of the cases of intestinal obstruction with albuminuria the albumin disappeared after the obstruction was relieved even though the bowel had become gangrenous. The cause in these cases of the albuminuria is uncertain but it certainly was not any attending peritonitis.

Albuminuria with Definite Renal Lesions.—An active renal congestion such as that due to exposure to cold, or in children to the acute infections, and chronic passive congestion due to heart or lung disease, to tumors, or to pregnancy, usually produce an albuminuria. The albumin in such cases is small in amount and runs parallel to the amount of urine, while in cases of true nephritis its percentage varies inversely as the amount of urine.

NEPHRITIS.—All cases of nephritis at some time during their course produce an albuminuria and in general the more acute the nephritis the larger the percentage of albumin, but not the larger the total amount of albumin per day. For, to excrete a larger amount of urine with a lower percentage of albumin is evidence of a better renal condition than when the percentage is higher but the total output of urine and so of albumin is smaller. It is not true that the prognosis of the case is always determined by the acuteness of the nephritis. In very young persons this may be the case but in nearly all adults with so-called acute nephritis the kidneys suffer also from previous attacks of this disease, or from years' long continued attacks, which have led to extensive destruction of renal epithelium. In the renal disturbance present at any one time, the acute element, of which the albuminuria is a good index, may be relatively little. It is for this reason that in cases of chronic interstitial nephritis ending in uremia and death the urine may contain but mere traces of albumin. In severe cases periods of albuminuria may alternate with months during which the urine is quite clear of demonstrable protein. In some cases of definite acute nephritis there may be no albuminuria.[125] In nephritis the urine seldom contains more than 1% of albumin. Sometimes it contains 2%, in very rare cases 5%, and in 1 case 8%. Senator mentions a case of subacute nephritis whose urine, for a period covering several days, contained from 6 to 8% of albumin. These cases with large amounts of albumin are, interestingly, often luetic. There are between 20 and 25 such cases of nephritis syphilitica acuta precox on record. In Hoffmann's case the enormous albuminuria ran parallel to the luetic symptoms and improved under mercurial treatment. In amyloid disease of the kidney the amount of albumin may be great or very small. As a rule it varies from 0.5 to 0.05%.

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[124] Neumann, Trans. Clin. Soc. of London, 1897, Bd. 30, p. 65.

[125] Herringham, Trans. Clin. Soc. of London, vol. xxxiv, p. 901.

Salkowski reported a case [126] the urine of which had a specific gravity of 1.056 and contained 7% of proteid. On standing, a rich, white, amorphous precipitate, not a coagulum, was deposited which gave the chemical reactions of albumin. On another day the urine of this patient contained even 8.5% of albumin. (The blood contains only about 7.5% of proteid.)

The total daily output of albumin in cases of nephritis is usually small, from 1 to 20 gms. Nephritis is not serious because of the actual loss of albumin to the body, for this could as a rule easily be covered by a very slight increase in the diet.

The albuminuria of a case of nephritis can be judged accurately only if the patient is on a carefully controlled diet and if the curve of the daily albumin, water, and nitrogen output are followed for at least 3 weeks. The customary methods of urine examination and the clinical uses made of the results of these examinations show an amazing lack of intellectual honesty or of downright ignorance. These curves show marked waves due apparently to changes in the temperature of the room, its humidity, the barometric pressure, others due to the mental condition of the patients, others due to changes in the diet, etc., but all too complex for off-hand analysis. In some cases thus studied it would seem as though meat were not as harmful as vegetables, some cases seemed to improve if salts are prohibited, some if used. In most cases the albumin is decreased (other things practically equal) if the patient is on a milk diet abundant enough to cover tissue needs. In practically all cases the albumin is increased by the erect posture, for all cases of albuminuria are relatively orthostatic, but this does not explain its increase during the waking hours of those patients who because of hydrothorax or cardiac disease are propped up against a back rest at about the same angle day and night. Exercise of any kind, even massage (Edgren), increases the output. As a case improves the value of an increase in diet and an increase of exercise will be proven by a diminution of the albuminuria.

"Hetero-albumosuria;" Bence-Jones Body; "Kahler's Disease;" "Myelopathic Albuminosuria of Bradshaw;" Theromolytic Albuminuria.— A remarkable proteid formerly called "albumose" appears in the urine of certain patients in very large amounts. It was supposed because of some of its chemical properties to be an hetero-albumose. Magnus-Levy however, who obtained it in crystalline form, showed that among its digestion products are all the primary proteoses except hetero-albumose. It would seem to belong in a group by itself standing nearest the true albumins. The safest course is, therefore, to call it "Bence-Jones body." In 1903 but about 35 well studied cases had been reported, all but one of which (a patient with lymphatic leukemia (Askanasy)) were cases of multiple myelomata. Later Boggs and Guthrie [127] report the condition in a case of carcinoma with metastases to the bone-marrow. In all the cases reported there was extensive disease of the bone-marrow. The most of the patients died in less than 2 years from the discovery of the condition.

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[126] Berl. klin. Wochenschr., March 3, 1902.

The Bence-Jones body is present in the urine often in large amounts, even 7%, but in the majority of cases there is less than 1%. Some cases are reported as intermittent (Boston). Coriat [128] reported a case with none in the urine but with 4% in the pleural fluid.

Reactions.—If urine containing the Bence-Jones body is first acidified with acetic acid and then warmed there will develop at a low temperature (about 60° C., often 52°) a milky, then a heavy, sticky precipitate which will for the most part, and in some cases perfectly, disappear on bringing the urine to a boil, but which will reappear on cooling. This is the characteristic reaction, and suggested the name "thermolytic albuminuria" proposed by Hugounenq for this condition.

Another very important test is the following. If nitric acid be added to the urine a heavy precipitate forms, which is soluble on warming and reappears at cooling. The urine will give the biuret reaction.

These striking reactions should attract attention at once. The urine may be turbid when voided. The moderately low temperature at which the precipitate appears, in general below 60° C., depends on the amount of Bence-Jones body present and also on the salt content of the urine. The properties of the Bence-Jones body found have been described so differently that it might seem as though we were dealing not with one but with a group of bodies. The chances are, however, that these differences are due to the varying amounts of salts and urea in the urine.

Boston [129] proposed the following test for the Bence-Jones body, based on the large amount of loosely bound sulphur it contains. From 15 to 20 c.c. of urine are mixed in a test-tube with an equal amount of saturated NaCl and well shaken. Then 2 to 3 c.c. of 30% NaOH are added and the tube shaken hard. The urine at the top of the tube is then heated to boiling and PbAc solution (10%) added drop by drop, heating the urine after the addition of each drop. In ½ to 1 minute there will develop a brown, later a black, precipitate.

The daily amount of this body eliminated is quite constant and since it is not affected by diet it probably is not a non-assimilable product of digestion but rather a substance formed in the bone-marrow, some say from the granules of the myelocytes and tumor-cells.

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[127] Bull. of the Johns Hopkins Hosp., Dec., 1912.

[128] Am. Jour. Med. Sci., 1903, vol. cxxvi.

[129] Am. Jour. Med. Sci., 1902, vol. cxxiv.

This albumose can be demonstrated also in the ascitic fluid, blood and bone-marrow of patients with this disease.

Quantitatively the Esbach's tube method will give an approximate determination (see page 216).

Albumosuria, " Peptonuria."—Under the term albumose have been described at least 2 different groups of bodies, " Bence-Jones body," and several digestion products formerly called " peptones " (a name given by Brücke to proteids not precipitated by K_4FeCN_6 and acetic acid) but now identified as a mixture of the deutero-albumoses and the peptone of Kühne. (Kühne defined a peptone as a proteid not precipitated by complete saturation with $(NH_4)_2SO_4$ and yet which gives the biuret test.) The true peptone (of Kühne) always with albumose (the reverse is not true), has been identified in the albumosurias of croupous pneumonia, of ulcer of the stomach, of pulmonary tuberculosis and of the puerperium.[130]

In testing for the deutero-albumoses the urine should be made albumin-free by the Hofmeister method, Mörner's body removed by basic lead acetate and the urine then saturated with ammonium sulphate. A flocculent precipitate indicates albumose (Hofmeister). A better method is to add to the urine (cleared of albumin and of Mörner's body) ⅕ volume of concentrated acetic acid and then phosphotungstic acid. The precipitate is dissolved in a little water, NaOH or KOH are added in excess and then very dilute $CuSO_4$. It may be necessary to filter off the precipitate of $Cu(OH)_2$. Deutero-albumoses are indicated by the appearance of a violet-red color.

Hammarsten recommends a method which, as modified by Bang, is of clinical value. Ten parts of urine plus 8 parts of saturated ammonium sulphate are boiled for a few seconds and this hot fluid then centrifugalized from ½ to 1 minute and decanted. The urobilin is then extracted from the precipitate with alcohol, the residue then taken up with little water, heated to boiling and filtered. In this way the albumin is removed. The filtrate is then shaken out with chloroform to remove the last trace of urobilin, the chloroform pipeted off and the biuret test applied to the remaining aqueous solution.

Alder [131] recommended the following test as more accurate. Albumin if present is removed by trichloracetic acid (15%). To from 6 to 10 c.c. of urine in a test-tube are added 1 or 2 drops of HCl till acid, then 5% phosphotungstic acid till precipitation is complete. The fluid is then centrifugalized for a few seconds. The supernatant fluid is poured off, the sediment suspended in absolute alcohol and again centrifugalized. This is repeated till the sediment and the alcohol (colored yellow with urobilin) are white and clear. The sediment is then suspended in water, strong NaOH added, the fluid shaken till all blue color disappears, then $CuSO_4$ solution is added. By this method even 0.2 gm. of albumose per liter can be detected.

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[130] Ito, Deutsches Arch. f. klin. Med., 1901, vol. lxxi.
[131] Berl. klin. Wochenschr., 1899, pp. 764, 780.

The deutero-albumoses may appear in the urine alone or with albumin. Albumosuria is as a rule clinically important only if the urine is quite free of albumin. In nephritis, albumose is often present with the albumin, but it may precede it or continue after the albumin has disappeared. Its presence in nephritis formerly was interpreted as due to the products of digestion which escape through the kidneys but now is attributed to a pepsin-like ferment which is often present in the urine. The albumosuria often present in luetic nephritis may arise in gummata undergoing involution.

HEMATOGENOUS ALBUMOSURIA.—When there is considerable albumose in the blood some at least will be excreted in the urine, but not if the amount in the blood is small. Albumosuria may therefore accompany any condition with disintegration of a tissue or exudate, or any disease with increased catabolism, as cancer or the fevers. It occurs therefore in leukemia, scurvy, purpura, in cases poisoned by a hemolytic poison or by a toxin which destroys tissue-cells. The albumosuria of the puerperium is ascribed to the involution of the uterus; that following the death of a fetus to the maceration of the infant; but albumosuria occurs also in some cases of normal pregnancy.

ENTEROGENOUS ALBUMOSURIA develops in some cases of gastric or intestinal ulcer, as, *e.g.*, in intestinal tuberculosis. In such cases the ingestion of small amounts of certain albumoses, *e.g.*, of somatose, will be followed by an albumosuria, but usually large amounts must be administered (alimentary albumosuria). The attempt to make use of this as a test of ulcer has not been very successful. (The patient was fed from 40 to 60 gms. of albumose. If an albumosuria followed the presence of a gastric or intestinal ulcer was assumed.)

"HEPATOGENOUS ALBUMOSURIA" develops in acute yellow atrophy, in cirrhosis of the liver and cancer of the liver, in catarrhal jaundice and in phosphorus poisoning. "Febrile albumosuria" is met with in the infectious fevers as the temperature falls, in rheumatism, septicemia, typhoid, phthisis, gangrenous processes, measles, scarlet fever, erysipelas and smallpox. It occurs in some mental diseases and in paralytic conditions. "Pyogenic albumosuria" is supposed to accompany the absorption of an exudate, as in pneumonia during resolution, in empyema, bronchiectasis, epidemic cerebrospinal meningitis, abscess and in osteomyelitis. It may be met with in gangrenous processes or cancers of any organ.

One should of course exclude the albumose of spermin and that of the secretions of the accessory genital glands.

Since albumosuria is met with in so many conditions it can have relatively little clinical value, yet it may be of some service in the recognition of a suspected abscess (*e.g.*, of the appendix, brain, or an empyema) and in the differential diagnosis between tuberculosis and epidemic cerebrospinal meningitis.

Hematuria.—Hematuria may be met with under the following conditions:

(1) General diseases: the malignant forms of acute specific fevers, especially smallpox, typhoid fever and malaria; in leukemia occasionally; in the so-called hemorrhagic diathesis, as hemophilia, scurvy, morbus maculosus Werlhofii and the purpuras. In the latter diseases the process may even be limited to the kidney.

(2) Renal causes: acute and chronic passive congestions and inflammations of the kidney; all forms of nephritis at the onset and practically every form of nephritis at some time during its course; nephropathies due to turpentine, carbolic acid and cantharides, especially at the onset; subacute parenchymatous nephritis always, Weigert said; in renal infarctions, although marked hemorrhages are rare; in new growths of the kidney, in which cases the hematuria sometimes is profuse; at the onset of renal tuberculosis, especially if the papillæ are involved; in cystic kidneys, renal calculus and, lastly, in parasitic diseases of the kidney, especially that due to filaria, echinococcus and Distoma hematobium. In congestion due to venous thrombosis, *e.g.*, of the new-born, hematuria is said to be common.

(3) It may be due also to lesions or diseases of the urinary passages: *e.g.*, stone in the pelvis or ureter, tumors and ulcers of the bladder, parasites of the bladder, calculi, ruptured veins and urethritis.

(4) In trauma of any part of the urinary tract from the kidney down.

(5) And, lastly, there is an interesting group with no known lesion, the so-called "Gull's renal epistaxis," "essential renal hematuria," "angioneurotic hematuria," or "renal hemophilia." This is a rare disease of middle adult life. The hemorrhage in these cases may be unilateral. In a few of these cases angiomata of the kidney have been found, in others no gross lesions and so nervous causes are suspected. Some of these cases recover without any special treatment, others after treatment for a neurosis, while others after a nephrotomy, a nephropexy or the simple exposure of the kidney.[132] Eshner [133] collected 48 cases of unilateral renal hematuria, most of which had been diagnosed as calculus or cancer. Since then other interesting cases have been reported.

Recent more careful examinations have thrown considerable doubt on the normal condition of these kidneys and only too often has the diagnosis of "essential hematuria" been the confession of ignorance and done great harm. A diagnosis of unilateral hemorrhagic nephritis was made in Stich's case.[134] In Schüller's [135] case the kidney looked normal on gross examination, but microscopically a chronic parenchymatous nephritis was demonstrated.

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[132] Stavely, Johns Hopkins Hosp. Bull., March, 1893.

[133] Am. Jour. Med. Sci., 1903, vol. cxxv.

In conclusion it may be said that the most frequent causes of profuse urinary hemorrhage are stone, tumor and tuberculosis, and in cases of profuse painless hemorrhage tumor especially must be excluded.[136]

It is customary to limit the term " hematuria " to those conditions with the blood grossly visible in the urine. Such urine is always turbid and has a color which varies from a light smoky tint to a bright red or blackish-brown. Microscopically the red blood-cells are found in various states of preservation, as well as other elements which will vary according to the cause. In renal hematuria the blood seldom clots, but is homogeneously mixed with the urine while in cases of vesical hemorrhage the second glass of the 2-glass test will contain more blood than the first. If in a case of bleeding from the bladder this organ be irrigated, all the washings will be blood-stained, while in renal cases some will be clear. In the acute exacerbations of a subacute parenchymatous nephritis especially the amount of blood in the urine may be considerable. Blood clots are passed in cases of trauma, aneurism, or varices, and in cancer more often than in calculus. Sometimes the shape of the clot will betray its origin in the renal pelvis or the ureter.

Gerhardt thinks that if the hemorrhage is from the renal cortex the red cells in the urine are more apt to be fragmented or more spherical and more leathery in color than usual, and will be accompanied by casts of various kinds, especially blood-casts, or casts with red cells attached, and renal epithelium.

In women the vagina must always be excluded as the source of the blood.

Hemoglobinuria or the presence of free hemoglobin in the urine will develop whenever the destruction of red blood-cells is so great that the body cannot warehouse the liberated hemoglobin. This will be the case when about $\frac{1}{60}$ or more of the total hemoglobin is set free at one time. Such a condition may be due to various blood poisons, as potassium chlorate, pyrogallic acid, CO, naphthol, AsH_3, etc.; or to the poisons of fevers, especially malaria and lues, but also scarlet fever, typhoid, yellow fevers, etc.; or to severe burns, exposure to cold, or the transfusion of an incompatible serum. It may occur during pregnancy (Brauer), as an epidemic fever of the new-born, in certain cases of nephritis and after severe intra-abdominal hemorrhages.

The best known form of hemoglobinuria is the black-water fever seen in malarial countries. Curry, Brem [137] and others have shown that while many cases of this condition are quite certainly due to malaria, yet it is not always possible to prove this by demonstrating malarial parasites either in the blood or the internal organs. Black-water fever may recur later after an average dose of quinine.[138] In this disease the hemoglobinuria is always accompanied by an intense albuminuria. These may appear synchronously, but the albuminuria may persist after the hemoglobinuria has disappeared (Brem). It is the general belief that a hemoglobinuria is always the result of a hemoglobinemia, although this has never been proved in the hemoglobinuria of infectious diseases or of hemorrhagic nephritis.

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[134] Mitth. aus. d. Grenzgeb. d. Med. et Chir., 1904, Bd. 13, p. 781.

[135] Wien. klin. Wochenschr., 1904, No. 17.

[136] Kretschmer, The Jour. of A. M. A., Feb. 24, 1917, lxviii, p. 578.

[137] Jour. Am. Med. Assoc., May 3, 1902; Brem, Jour. of A. M. A., Dec. 8, 1906; Lovelace, Arch. of Int. Med., June 15, 1913, vol. xi, p. 674.

Paroxysmal hemoglobinuria is an interesting and rare disease of adults the chief symptom of which is an hemoglobinuria following exposure to cold or exertion, often preceded by fever, with chills and pain in the lumbar regions, which may last for a few hours or even for 2 days. This hemoglobinuria is usually preceded by a hemoglobinemia.

The urine in this condition, which should be examined while very fresh, may be clear but it is usually more or less clouded by hemoglobin casts, amorphous masses of pigment and by casts due to the associated nephritis. When centrifugalized, the supernatant urine is a clear, blood-colored fluid, while the sediment contains very few red blood-cells but amorphous blood-pigment in masses or casts, and even crystals of hematoidin. Often hyaline and granular casts and renal epithelium are present and sometimes many calcium oxalate crystals. The urine of a case of hematuria not examined fresh may contain much hemoglobin in solution since the red blood-cells will quickly lake but the sediment of such a specimen will be abundant, grayish-brown in color and in it may be seen the stromata of the many laked red cells.

With the spectroscope one will find in the urine of a case of paroxysmal hemoglobinuria methemoglobin alone or this together with hemoglobin. Serum albumin is always present in the urine and often bile pigment, but, it is said, never any bile acid. After the hemoglobin disappears the albuminuria may continue for a short time. Other features of the blood during an attack are a leucocytosis and a great increase of the number of platelets. Sometimes the hemoglobinemia is evident from inspection of the plasma of a specimen of centrifugalized blood.

Among the common immediate causes of the attacks of paroxysmal hemoglobinuria are excessive exercise and mental excitement but exposure to cold is the most important. Some patients can induce an attack by plunging the hand into cold water and Homburg's patient [139] showed it after an involuntary cold plunge of 3 minutes duration. It may be produced locally by tying a string about 1 finger. Some claim that the cause of these attacks of hemoglobinemia is the hemolytic action of the patient's own blood-plasma;[140] others, a chemical toxin; others, an increased susceptibility of the cells to mechanical injury (and in the circulation shadows are found); others, including Senator, think the cause is in the kidney (see page 328); others say lues and it is of interest that at least 23 of the 77 cases reviewed gave a history of lues: but the best explanation is that of Moss (see page 529).

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[138] Nansen, Brit. Med. Jour., May 16, 1903.

[139] Zeitsch. f. klin. Med., vol. liii.

[140] Hoover and Stone, Arch. of Int. Med., Nov., 1908, vol. ii, p. 392.

Tests for Hemoglobin in the Urine.—Whether or not hemoglobin is still within its cells the microscope alone can decide. To determine the chemical form of hemoglobin and its modifications, spectroscopic examination is necessary. The chemical tests are the same for hemoglobin and all of its modifications.

(1) The presence of blood in the urine may be suspected if the coagulum formed by the ordinary heat-acetic-acid test for albumin is brown, swims on the surface and is decolorized when shaken out with alcohol acidified with H_2SO_4. This test is not very delicate.

(2) *Heller's Test.*—The urine, which half fills a test-tube, is made strongly alkaline with about 5 drops of NaOH and then warmed in order to transform all hemoglobin present to hematin. The precipitate, consisting of the phosphates and carbonates of the alkaline earths, which form under these conditions will carry down all the hematin and therefore have a brownish red or blood-red color. If the urine originally used was already alkaline the phosphates of the alkaline earths may already have precipitated, in which case it is necessary to add a certain amount of normal urine in order to supply these salts.

This test is very delicate, indicating as it does 1 c.c. of blood in 1 liter of urine. If the fine red color of the precipitate is masked by the dark color of the urine or by bile the precipitate should be filtered off and dissolved in acetic acid. A red solution is obtained which will decolorize gradually in the air. This red precipitate has by reflected light a greenish tinge. If but little hematin be present the precipitate should be freed of all inorganic salts by dissolving them in acetic acid and the residue used for Teichmann's test. Precipitates similar in color may be obtained after the ingestion of senna, rhubard, or rhamnus. The urine in these cases, however, should be yellow at first and red on the addition of sodium hydrate. Hematoporphyrin and other pathological pigments may give a red precipitate, but the spectroscope will quickly indicate the difference, for if blood was present we now would get the spectrum of alkaline hematin.

If but a trace of blood is present the urine may first be made alkaline with NH_4OH and then precipitated with tannic acid. The precipitate obtained is used for the hemin crystal test.

(3) *Teichmann's HCl-Hemin Test.*—The precipitate obtained by either of the preceding methods, or, better still, a tannic acid precipitate, is filtered, washed and thoroughly dried in the air. A very small granule of this dry precipitate is put on a slide with 1 minute granule of NaCl and a few drops of glacial acetic acid and covered with a cover-glass. The speci-

men is then warmed over a small flame so that the acetic acid will just steam and not boil, renewing the acid as rapidly as is necessary. When the hot acetic acid surrounding the granule has taken on a brownish color, the heating is discontinued and the slide allowed to cool slowly. The characteristic crystals of hemin may soon be seen. Excellent specimens may be obtained without the use of heat if the specimen is allowed to stand for 24 hours (see Fig. 36).

This is a very good test to use as a class exercise since it trains the student in a valuable and much neglected technic.

(4) The tannic acid precipitate mentioned in test (2) may be ashed on a platinum foil, the ash dissolved in a few drops of hot HCl, this diluted and filtered and tested for iron with the potassium ferrocyanide solution.

(5) *The Guaiac test* (Schönbein-Almén test) is very delicate. The urine acidified with acetic acid if necessary, is overlaid carefully with a mixture of equal parts of guaiac tincture (alcoholic solution of resina guaiaci, 1 to 5) and old oxygenated oil of turpentine. The turpentine should previously have been exposed to the air for some time that it may be well oxygenated; the guaiac tincture should be kept in a colored bottle and not unnecessarily exposed to the sun or air. These solutions when mixed should not develop any trace of a blue color. If blood be present an intense blue ring will develop at the line of separation.

This test is so delicate that it may detect blood even when the spectroscopic test is negative. Pus will not lead to error if the solutions have been properly kept. A control should always be made with a fluid known to contain blood. While certain other bodies sometimes present may give a positive test yet a negative result always means that no blood is present.

FIG. 36.—Hemin crystals. × 400.

The most delicate of all tests for blood in the urine is the phenolphthalein test of Kastle.[141] This is said to show 8 parts of blood in 1,000,000 parts of urine.

Spectroscopic Tests.—The spectroscopic examination of a urine which contains blood will usually show a mixed spectrum. If the blood is fresh the lines of oxyhemoglobin will predominate, but in cases of hemoglobinuria or of nephritis, those of methemoglobin will be conspicuous. Bacteria will tend to oxidize this lack back to oxyhemoglobin. The urine used must be clear and should if necessary be diluted.

Very small amounts of blood-pigment may be detected as follows (Hoppe-Seyler): To 100 c.c. of the urine is added a solution of albumin or an albuminous urine. The urine is then coagulated by heat, filtered, the precipitate washed, pressed out and rubbed up with alcohol which contains a little H_2SO_4. It is then warmed and filtered. The filtrate after the addition of NaOH and $(NH_4)_2S$ gives the spectrum bands of hematin.

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[141] Bull. 51, Hygienic Lab., Public Health and Marine Hospital Service.

METHEMOGLOBIN.—Many previous reports of the presence of methemoglobin in the urine are not reliable since hematin was not excluded. Methemoglobin is present in all fresh urines containing blood, although in time the bacteria may oxidize it to oxyhemoglobin. The spectrum of neutral methemoglobin resembles closely that of hematin, but if ammonia be added that of alkaline methemoglobin will appear. This spectrum may be confused if other bodies which either have a spectrum of their own or which darken the field, as bile or urobilin, also are present. One must be careful not to dilute the urine too much for this test.

Urobilin and bile-pigment may be removed from the urine with basic PbAc, in which case hemoglobin will remain in solution, but methemoglobin will be precipitated. In case the hemoglobin is still present in red blood-cells, water should be added in sufficient amount to lake them. If the spectrum of hemoglobin cannot be seen or is very faint the hemoglobin may be transformed to reduced hematin whose spectrum is much easier to study. This reduction is best accomplished with $(NH_4)_2S$, or with $NaHSO_3$ and zinc. If the action of these reagents is prolonged albumin will be precipitated. The spectrum of reduced hemoglobin is fainter than is that of oxyhemoglobin.

HEMATOPORPHYRIN.—Hematoporphyrin, an iron-free derivative of hemoglobin, is present in normal urine in traces, but under certain conditions it may appear in very large amounts. The most important of these conditions are: the protracted use of sulphonal especially, but also of trional and tetronal; the acute infectious diseases, including acute rheumatic fever and pericarditis; the various forms of tuberculosis especially; Addison's disease; paroxysmal hemoglobinuria; pneumonia; lead poisoning; hematemesis; while some claim that an increase of this pigment in the urine is important in the diagnosis of liver disease, especially of cirrhosis. Pal[142] reported a case of paroxysmal hematoporphyrinuria due, he thinks, to lues, with "black" urine and with symptoms similar to those of paroxysmal hemoglobinuria.

Urines rich in hematoporphyrin usually have a color described as dark brownish-red, cherry-color, Bordeaux-red, or "Port-wine" color. This color is, however, sometimes deceptive, especially in the cases due to lead poisoning, since it is not the hematoporphyrin itself which explains the peculiar color. In fact, considerable of this pigment may be present in a normally colored urine, while all of the hematoporphyrin may be removed from a highly colored urine without changing its color and, finally, some urines whose color strongly suggests the presence of this pigment contain none at all. What the pigments are which are so often excreted with hematoporphyrin is not known.[143]

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[142] Centralbl. f. inn. Med., 1903, vol. xxiv, p. 601.

[143] Monro, Quart. Jour. Med., 1907, vol. i, p. 49; 1910, vol. iv.

About forty fatal cases of hematoporphyrinuria following the use of sulphonal have been reported. The most of these were women. Garrod [144] collected 12 cases not due to sulphonal, nearly all in men whose condition lasted for years without any bad symptoms.

The cause of hematoporphyrinuria is not known. Some claim that lead, sulphonal, etc., have a direct action on the red blood-cells; others, that they cause hemorrhages into the gastric mucosa and that the blood-pigment there liberated is changed by the gastric juice to hematoporphyrin which is absorbed and excreted; while in other cases the trouble is said to lie in the renal epithelium. An hematoporphyrinemia has not yet been proved.[145] Some ascribe the condition to perverted catabolism of hemoglobin rather than to increased destruction of red cells.

Salkowski's Method of Detecting Hematoporphyrin in the Urine.—From 30 to 50 c.c. of urine is completely precipitated by an alkaline solution of barium chloride (equal parts of a cold saturated solution of barium hydroxide and of a 10% solution of barium chloride) and filtered. The precipitate is washed, first with water and next with absolute alcohol. It is then extracted by pouring repeatedly warm acidified alcohol (10 c.c. of alcohol containing from 6 to 8 drops of HCl) over the precipitate on the filter paper. If hematoporphyrin is present the alcoholic filtrate will become reddish-violet in color, due to acid hematoporphyrin, and its spectrum will show two absorption bands. If now ammonia be added the color of the solution will change to yellow and the spectrum will show the 4 bands of alkaline hematoporphyrin (Sahli).

SEDIMENTS

Preservation of the Urine.—To study organized sediments the urine should be examined while perfectly fresh, for casts often disintegrate rapidly. The best way to get the sediment is to allow the urine to stand in a conical glass in a room at low temperature. The most convenient way, however, is to centrifugalize the fresh urine in a centrifuge. One great disadvantage of this method is that the high pressure at the point of the tube may compress casts, cells and mucus into an indistinguishable mass. A quick but not accurate way is to filter the urine through a filter paper and examine the last few drops in the funnel. A drop of the sediment is drawn up from the point of the conical glass, centrifuge tube, etc., into a clean pipet; the outside of the pipet is wiped off and the drop blown onto a large, clean glass slide. No cover-glass is necessary for the preliminary survey of the field by low magnification, but a large cover-glass should later be dropped over interesting positions of the field and the higher dry powers used. If the urine contains very much sediment this will settle in layers, the composition of which will vary considerably. Such a sediment should first be well mixed or the several layers separately examined.

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[144] Lancet, March 5, 1904.

[145] Ruedy, Am. Jour. Insanity, October, 1899.

To preserve the urine from bacterial action during long sedimentation a piece of camphor or of thymol or a few drops of formalin are best, but the latter may add to the sediment a component of its own. Chloroform, so valuable in the preservation of crystals and for chemical work, cannot be used to preserve casts and cells.

To preserve sediments for long periods of time the urine is centrifugalized and the supernatant fluid poured off. Chloroform is then added to preserve crystals, or formalin to 1 to 2% to preserve casts and formed elements.

Since there are very few inorganic sediments which can be recognized beyond doubt from their appearance alone the student should practise the methods of microchemistry. The reagents may be drawn under the cover by applying the edge of a piece of filter paper to the edge of the cover-glass opposite to a drop of reagent used which just wets the edge of the cover-glass.

There is one peculiarity of crystalline sediments worthy of mention, that the crystals of any one substance in any one urine all usually belong to the same system. Another peculiarity is the relative infrequency of crystalline sediments in women's urine.

The sediments have been divided into those which are ORGANIZED and UNORGANIZED. These terms are relics of an antiquated pathology. By the former are meant tissue elements, casts, bacteria, and formed elements from the urinary or communicating organs; by the latter, any precipitate. They may also be divided into gross and microscopic sediments. The normal urine when passed never has a gross sediment but always a microscopic organized sediment consisting of a trace of mucus and a few mononuclear cells. The uncatheterized specimen of a woman's urine will practically always contain also a few cells from the genital tract which are washed by the stream of urine from the mouth of the urethra and the labia. After the urine has stood for even a few minutes crystals or a gross amorphous sediment may appear, the amount, composition and character of which will depend on the temperature, the concentration and reaction of the urine and on the rapidity of ammonia production. It will depend much less on the diet.

A gross sediment of phosphates and carbonates of the alkaline earths may cloud the urine when passed in cases of phosphaturia, persons whom we must consider normal. After a normal urine, perfectly clear when voided, has stood for a shorter or longer time, an inorganic precipitate, often abundant, may appear, of uric acid or urates in an acid urine and of phosphates and carbonates in an alkaline urine. In pathological conditions the organized sediments often are gross. These may consist of blood, pus, cystin, even of casts (see page 272) while an inorganic alkaline phosphate sediment may be voided with the urine in various diseases of the kidney and urinary tract.

The reaction of the urine may often be indicated by the appearance of a gross sediment; when acid, this is granular; when alkaline, it is mucoid. Preparations for microscopic study should be examined as soon as made, since if allowed to dry even a little the crystals or urea, NaCl, etc., which separate, may confuse one.

Unorganized Sediments—(1) Urates and Uric Acid.—The urates may separate from any normal concentrated acid urine, especially on a cold day (much to the distress of mind of some persons) first as a remarkably muddy or milky cloud, then as a heavy voluminous sediment, the color of which will vary from a yellow to a bright rose-red, which settles on the bottom and sides of the container. It disappears at once on warming. This is the most characteristic reaction. It is soluble in acids, with the subsequent precipitation of uric acid, and in alkalies. This sediment is especially common in the urine of patients with fevers, *e.g.*, pneumonia, acute rheumatic fever and in chronic passive congestion. It rarely forms in albuminous urines.

This precipitate is said to be composed of the quadriurates (Roberts), $MH\bar{U}\bar{U}$, which are said to be formed by the action of MH_2PO_4 on the biurates, $MH\bar{U}$. If present in sufficient concentration the quadriurates will precipitate as such, but if present in less-concentrated solution they will be decomposed, forming biurates and uric acid, the latter of which will at once precipitate as little, bright red, so-called "red pepper granules" on the sides of the glass. In the urate sediment are found also calcium oxalate crystals and, as ammonia soon forms, ammonium urate globules. Hence in the same sediment one may find ammonium urate, the so-called quadriurates, uric acid, calcium oxalate and even a few triple phosphate crystals. This transformation of the sediment progresses from above downward.

This explanation of Roberts would be very satisfactory were it not that it has little evidence behind it. One thing is quite certain, that the precipitation of the urates is the result of a chemical and not merely a physical change in the urine. The precipitate forms too slowly in the cooling urine to be due to temperature changes alone and warming the urine to its original temperature does not redissolve this sediment. Also, during its formation, the acidity of the urine is said to increase.

Microscopically, the acid urate sediment consists of clusters of very fine granules, the color of which varies from yellow to a reddish-brown, which disappear on warming. The addition of a little acetic acid will be followed by the appearance of uric acid crystals.

Ammonium Biurate.—Ammonium biurate (see Fig. 37) is the only urate sediment which forms in an alkaline urine. It may indeed begin to appear while the urine is still very faintly acid as soon as enough ammonia is available, it will increase in direct proportion as ammonia is formed and later will be mixed with a sediment of amorphous phosphates and triple phosphates. Microscopically it may consist of spheres which often present the so-called " morning star " or " thorn apple " shape, which have a dark color, are often concentric or radially striated and are covered with crystals projecting like thorns; but more often it assumes very irregular, bizarre shapes. This precipitate is soluble in acetic acid with the subsequent precipitation of uric acid and the liberation of ammonia.

Uric acid and the urates when pure are colorless but their common yellow color is due to urochrome especially, but also to urobilin, and the red to uroerythrin. The precipitating urates have a peculiar affinity for bile pigments and so this sediment may carry down and contain all the bile that there is in a specimen of urine. The same may be true of the black pigment of the urine in cases of carbolic acid poisoning.

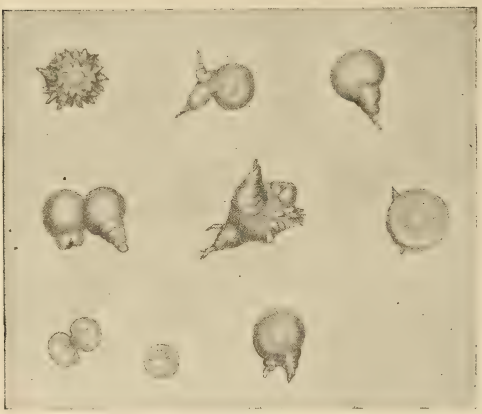

Fig. 37.—Various forms of ammonium biurate crystals. × 400.

The needle crystals of sodium biurate are rarely found in the urine and then only in urines undergoing ammoniacal decomposition but which are still amphoteric. These crystals resemble calcium phosphate, but when brought into contact with acetic acid they at once dissolve and a cloud of uric crystals appears.

Uric Acid.—Uric acid (see Fig. 38) when pure crystallizes usually in rhombs, but in the urine rhombs with definite angles are rarely if ever seen for the angles are rounded and the crystals have the so-called "whetstone" shape. When seen on the edge these crystals are very narrow rectangles. They may be single, in rosettes, or clustered in the shape of a barrel (see Fig. 38, *a*, *b*, *1*).

Sometimes the uric acid crystallizes in needles arranged in sheaves (see Fig. 38, *4*). These crystals may occur as masses as large as the head of a pin, which cling to the glass (see Fig. 38, *2*). Their color varies from yellow to brown, or they may be colorless. The colorless crystals are sometimes perfect hexagons in which case their recognition is difficult, since they may in appearance perfectly resemble cystin crystals. Dr. T. B. Futcher once provided our class in clinical diagnosis a remarkable specimen of urine which illustrates this point. The patient, a girl six years of age with diabetes mellitus, had been voiding from 1000 to 2000 c.c. of urine per day, with a specific gravity about 1.035; sugar, from 5.1 to 5.5% and nothing of interest microscopically. One day after 24 hours on a carbohydrate-free, proteid-rich diet the urine (its sp. gr. was 1.026 and the sugar, 0.6%) was turbid because of a suspension of glistening, colorless, hexagonal crystals which in appearance exactly resembled cystin. Many were single, but most were in clumps of even macroscopic size. It was only on chemical examination that they could be recognized as uric acid crystals. On this day no typical uric acid crystals were seen. The following day the sediment consisted of a mixture of hexagonal and whetstone crystals and after that not a single hexagonal form was found.

The yellow color of uric acid crystals is due to urochrome, not to urobilin, and the red to uroerythrin plus urochrome. Hematoporphyrin, bilirubin, or biliverdin may if present give their color to these crystals. In cases of carbolic acid poisoning they may have a dark brown, almost black, color.

Crystals which are precipitated artificially by the addition of an acid to a urine have a reddish-brown color due to black decomposition products of urochrome; their color may also be due to indigo-blue or indigo-red, if these are present in the urine.

Calcium urate crystals are said to appear sometimes in the same sediments with calcium oxalate crystals. They are colorless prisms, insoluble in hot water, give the murexid test and are soluble in acid with the subsequent appearance of uric acid crystals. They may be produced by treating an acid urate sediment with lime water.

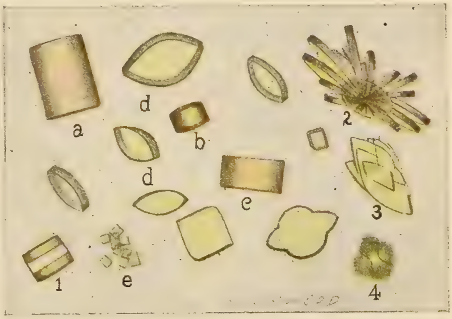

FIG. 38.—Uric acid crystals. (The lettered forms are drawn from nature, those numbered are copied from Rieder's Atlas.)

DETECTION.—The urate sediments which are deposited in acid urine may usually be recognized from their gross appearance alone, but the characteristic tests are that they disappear on warming and that they all are dissolved by acid with the subsequent precipitation of uric acid. Uric acid crystals are not dissolved by heat or by acid. Ammonium biurate spheres are characteristic in form and are soluble in acid followed by the appearance of the uric acid crystals.

Murexid Test.—This test is characteristic of uric acid and its salts. The crystal or sediment to be tested is evaporated in a porcelain dish with dilute HNO_3, and to the residue is added some weak NH_4OH. If uric acid (or urates) is present a beautiful purple-red color will appear.

A urate sediment has little significance except that it indicates a concentrated acid urine. A uric acid sediment, however, may have great importance since it sometimes forms large concretions in the pelvis of the kidney or bladder.

Phosphates and Carbonates.—(1) AMORPHOUS EARTHY PHOSPHATES AND CARBONATES may be precipitated in any urine by the addition of a little fixed alkali. A somewhat similar precipitate forms when a weakly acid or alkaline urine is heated, since the acid salts of phosphoric and carbonic acid are then changed to insoluble basic salts. Both are soluble in acetic acid, the carbonates with gas evolution. They are the chief constituent of the sediment of an alkaline urine and may cloud even the fresh urine of certain nervous cases and of cases of gastric hypersecretion who lose much acid from the stomach because of vomiting, lavage, or diarrhea. The total amount of phosphoric acid in the urine of these cases of so-called phosphaturia is not increased. Microscopically this precipitate is seen to consist of very coarse colorless granules varying considerably in size, which disappear on the addition of a little acetic acid. By the gas bubble formed one can tell which granules were the carbonates.

(2) TRIPLE PHOSPHATES, $MgNH_4PO_46H_2O$.—The beautiful triple phosphate crystals (see Fig. 39) appear in urine even while still acid as soon as sufficient ammonia is present. They appear usually in connection with the amorphous carbonates and phosphates, often with ammonium urate, while in some urines they are the chief constituent of the sediment. These crystals belong to the rhombic system. In size they vary from those very small to some even 9 mm. in length. The so-called coffin-lid crystals are characteristic (see Fig. 39), but many modifications of this shape may be found. Other strange X-forms are due to the partial solution of a crystal. Some are said to resemble calcium oxalate crystals but this resemblance is only superficial, for even when triple phosphate appears as a perfect pyramid with a square base there is no trace of a double envelope appearance (see Fig. 39 and page 251).

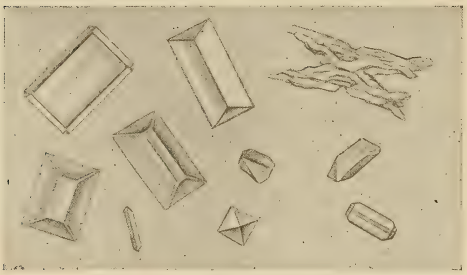

FIG. 39.—Various forms of triple phosphate crystals. × 400. To the left are coffin-lid shapes; in the lower center a perfect pyramid; that in the upper left corner resembles neutral magnesium phosphate; that in the upper right is a partially dissolved crystal.

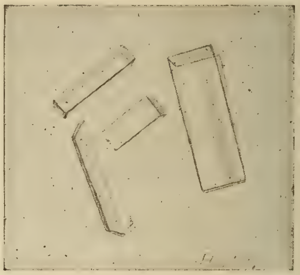

FIG. 40.—Atypical forms of triple phosphate crystals. × 400.

Fern-shaped crystals occur especially in sediments artificially precipitated.

In some urines nearly all the triple phosphate crystals have unusual shapes; some are very thin plates (see Fig. 39); some have bevelled edges, some apparently not; some have square, others rounded or bevelled corners, some are wedges (see Fig. 40), some triangular prisms; yet all have a greenish hue which is not seen in the calcium oxalate crystals.

Neutral Magnesium Phosphate, $Mg_3(PO_4)_2 22H_2O$.—The very rare crystals of neutral magnesium phosphate (see Fig. 47, *b*) have been found in alkaline urines in which the amount of ammonia was not sufficient to form triple phosphates. Such happens in certain cases of dilated stomach with considerable vomiting and also after the ingestion of magnesium carbonate, etc. These crystals are exceedingly refractile, long rhombic tablets with bevelled edges. Some resemble the very thin coffin-lid triple phosphate crystals (see Fig. 39). This is a beautiful sediment to study.

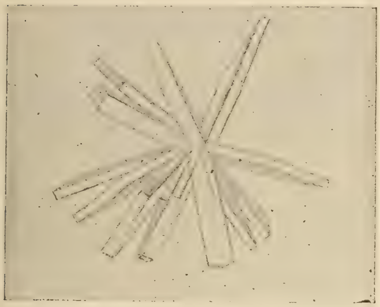

Fig. 41.—Wedges of dicalcium phosphate.

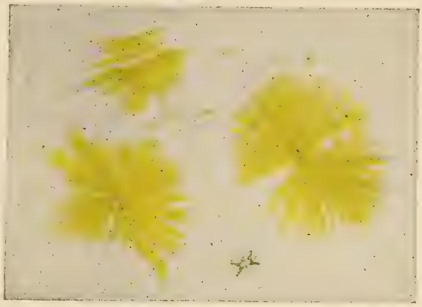

Fig. 42.—Sheaves of calcium phosphate needles. × 50.

Dicalcium Phosphate.—Dicalcium phosphate crystals are sometimes but rarely found in amphoteric or weakly acid urines. They are small prisms or wedges arranged in irregular clumps (see Fig. 41), or massed together in rosettes (see Fig. 47, *d*) or fan-shaped clusters. In these masses or rosettes the individual small crystal can hardly be made out. A rather unusual form of calcium phosphate crystal is shown in Fig. 43 and a still more unusual form in Fig. 42. Dicalcium phosphate may crystallize out when the urine is rich in calcium and only weakly acid, which would seem to occur especially in chronic arthritis. They are soluble in acetic acid. They may be distinguished from triple phosphates crystals since 20% ammonium carbonate will dissolve the dicalcium crystals and not the latter.

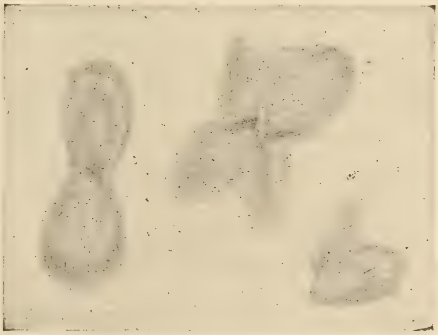

Fig. 43.—Calcium phosphate (?). × 400.

Calcium Carbonate.—These crystals (see Fig. 44) may be mingled with the amorphous carbonates in an alkaline urine. They occur as amorphous masses or as dumb-bells resembling somewhat the CaOx crystals, or as large concentric radiating spheres. They are soluble in acetic acid with gas liberation.

Neutral calcium phosphate may appear as a scum on the surface of even quite fresh urine, resembling a film of oil which may easily be skimmed off. This scum under the microscope is seen to consist of amorphous matter in sheets. This precipitate often is a nuisance since it clings to the outside of a pipet used for obtaining samples of the sediment.

Oxaluria.—The symptom complex, oxaluria, formerly so respected, is seldom mentioned now. This term was used of any nervous condition if the urine of that patient contained a large sediment of CaOx crystals. The precipitation of these crystals, however, does not depend as much on the total amount of oxalic acid present as it does on its solubility. The precipitation of CaOx in the urinary tract, on the other hand, is of very great importance since from 30 to 50% of urinary calculi consist of CaOx and these are the worst of stones.

While a certain amount of the CaOx of the urine is a product of tissue combustion, since some is present in the urine even of a starving person, yet the chief source is the food, especially certain vegetables as beans, artichokes, beets, potatoes and especially tomatoes, spinach, rhubarb, certain fruits and grains, cocoa, tea and coffee. Only about 15% of the oxalic acid ingested is absorbed into the bloodstream and this is excreted quantitatively in the urine as CaOx; about 10% of that ingested appears in the stools; the rest is destroyed by the intestinal bacteria and ferments.

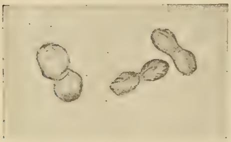
Fig. 44.—Calcium carbonate. × 400.

In health the output in the urine averages about 20 mgms. per day; the upper limit, 35 mgms. Bakhoven thinks that the carbohydrates of the foods are the chief oxalate builders. In its excretion oxalic acid bears no relation to the uric acid output; the latter, for instance, can be increased by a diet rich in the nucleins and there be no change in the oxalic acid output.

Calcium oxalate crystals, present in very fresh urines, attracted considerable attention of the older pathologists, as they were supposed to cause an irritation in the urethra and so explained many of the symptoms and vicious habits of neurotic individuals, *e.g.*, masturbation.

Among the diseases claimed to be accompanied by oxaluria are pulmonary tuberculosis, peritoneal tuberculosis, pernicious anemia, leukemia (in which condition, it is claimed, the output varies from 33.2 to 53 mgms. per day), jaundice, diabetes mellitus, gout, diseases of the digestive and respiratory organs, cirrhosis of the liver and, especially, neurasthenia. The amount of oxalic acid in the urine bears some relation to the absence of HCl in the gastric juice and to fermentation processes in the intestine. In diabetes mellitus a large output is usually present, which increases as the sugar diminishes (vicarious oxaluria). Naunyn mentions three cases in which quantitative estimates of CaOx were made. One eliminated 0.8 gm., the second 1.2 gms. in 24 hours and the third 0.5 gm. per liter.

It is of interest that some insurance companies regard oxaluria as an early sign of nephritis. The best recent work on this subject is that of Serkowski and Mozdzenski [146] who showed by accurate methods that there is no apparent relationship between the amount of oxalic acid in solution and that in the sediment, or between the amount of oxalic acid and of uric acid in the urine. Its output does, however, run roughly parallel to that of the acid phosphates.

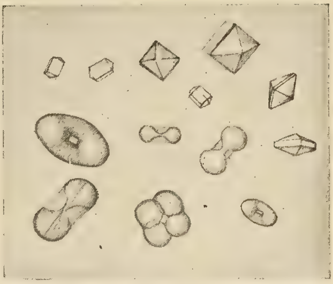

Fig. 45.—Various forms of calcium oxalate crystals and spheres. × 400.

Calcium oxalate crystals may precipitate in any urine but the real question chemically is, Why does any remain in solution? Klemperer and Tritschler[147] consider that the acid phosphates aid in holding it in solution, those of sodium least, those of calcium more and those of magnesium most; also that the absolute amount of CaOx in the urine is of importance. It is almost impossible to associate this sediment with any pathological condition.

CaOx crystals occur in 2 forms, the first of which, the double envelope octahedral form, is quite characteristic. On a hasty glance it may resemble some square triple phosphate crystals, but they are insoluble in acetic acid. The second form, the spherical, could be mistaken on hasty inspection for $CaCO_3$ crystals, although they show a different structure and are insoluble in acid.

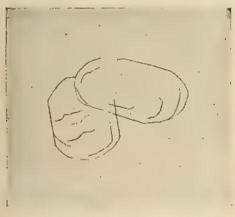

Fig. 46.—A rare form of calcium oxalate crystals.

(1) The octahedral ($CaC_2O_4 3H_2O$) which belong to the tetragonal system (see Fig. 45) resemble double envelopes or prisms.

(2) The spheroidal forms ($CaC_2O_4 4H_2O$) (see Fig. 45) are flat, oval, or nearly semicircular with a central groove and hence resemble an hour-glass. They often show a radial striation.

A rate form of oxalate crystal is represented in Fig. 46. These crystals were flat plates with parallel sides and rounded ends and resembled superimposed sheets of mica.

Calcium oxalate crystals are transparent, very refractive and usually colorless, but may be bile-stained if the urine contains bile pigment. They are insoluble in water, are very little soluble, if at all, in acetic acid, and are easily in any mineral acid. As this precipitate forms very slowly the crystals forms are perfect. They may be found in acid, amphoteric, or weakly alkaline urine, and are sometimes present in the specimen when voided.

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[146] Zeitschr. f. phys. Chem., Jan., 1911, vol. lxx, p. 264.
[147] Zeitschr. f. klin. Med., 1902, vol. xliv, p. 337.

QUANTITATIVE DETERMINATION OF OXALIC ACID.—The Neumann method is as follows: The 24 hours' collection of urine is precipitated with calcium chloride and ammonia and then made weakly acid with acetic acid. A small amount of alcoholic thymol solution is added to inhibit bacterial growth. The urine is now allowed to stand for over 24 hours in a warm place. The precipitate is then washed several times by decanting the supernatant fluid through a filter paper and finally bringing the entire precipitate onto the paper. As much of the washing as possible is done by decanting, since the fine precipitate passes easily through the paper. The precipitate is then dissolved in warm dilute HCl, and the paper washed with water until the water is no longer acid. This filtrate is evaporated to a small volume in a porcelain dish on the water-bath, then poured into a small stout cylinder, washing the dish with water and dilute HCl and adding the washwater to the fluid. Ammonia is then added in excess, a few drops of litmus being added to assure one of the reaction. After at least 24 hours standing, the precipitate is brought onto an ashless filter paper, loosening those crystals which cling to the walls of the cylinder with a glass rod the end of which is covered with a small piece of rubber tubing. The precipitate is then washed with water, until it is chlorine-free, and then with acetic acid. The filter is now dried, burned in a platinum crucible first at a dull red then with a blast flame until at constant weight. Calcium oxalate is thus transformed to calcium oxide, 50 parts of which correspond to 90 parts of oxalic acid.

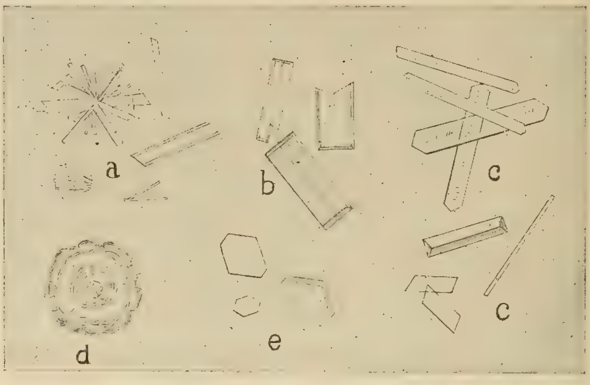

FIG. 47.—Various crystals: *a*, calcium sulphate; *b*, neutral magnesium phosphate; *c*, hippuric acid; *d*, acid calcium phosphate; *e*, colorless uric acid. (Copied from Rieder's Atlas.)

Calcium Sulphate ($CaSO_4 2H_2O$).—Calcium sulphate forms a very rare sediment found only in very acid urines. The crystals (see Fig. 47, *a*) are long, thin tablets or needles, some single but the most in clusters, which are insoluble in NH_4OH, alcohol, and acetic acid and soluble with difficulty in HCl, HNO_3 and in hot water (little in cold). The nature of these crystals should be confirmed chemically by dissolving them and proving the presence of sulphuric acid by the addition of a solution of $BaCl_2$.

Hippuric Acid.—Hippuric acid occurs rarely as a sediment of milk-white, semi-transparent, 4-sided prisms and rods with ends of 2 to 4 planes (see Fig. 47, *c*). These are distinguished from uric acid, which they may resemble in form, by their greater solubility in water, especially in warm water, their solubility in alcohol and ether and in that they do not give the murexid test. The amount of hippuric acid in the normal urine varies from 0.1 to 1 gm. per day, depending on the diet.

Hetero-albumose, Bence-Jones Body.—In 2 cases Bence-Jones body, once crystalline and once in amorphous condition, has formed a urine sediment.

Xanthin.—Two or three instances have been reported with xanthin crystals in the urine sediment. These crystals resembled uric acid somewhat (see Fig. 48, *d*) but are soluble on heating and in ammonia. If evaporated on a bath in quite concentrated NHO_3 they give a yellow residue, which color on further careful heating over a small flame becomes more intense. If now KOH is added the color becomes first yellowish-red, then a deeper red or even a violet-red. This test should not be confused with the murexid test.

Hematoidin (Bilirubin).—Hematoidin may appear as needles (Fig. 48, *a*) or rhombs (*b*) or in amorphous form in the urine sediment in hemorrhagic nephritis, in very jaundiced urine especially if acid has been added, in acute yellow atrophy, in pyonephrosis, with cancer fragments and after transfusion. In cases of jaundice of the new-born they may be found in the epithelial cells in the urine. They have also been found in connection with waxy kidney, scarlet fever, typhoid fever and carcinoma of the liver with jaundice.

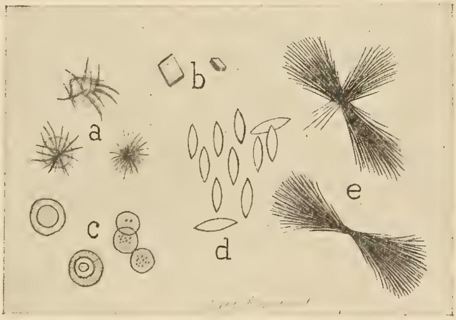

Fig. 48.—Various crystals of the urine: *a*, hematoidin needles; *b*, hematoidin crystals; *c*, leucin; *d*, xanthin; *e*, tyrosin. (Copied from various authors.)

Indigo.—Crystals of indigo, as a scum of blue needles arranged in stars or of blue rhombic plates which are soluble in chloroform giving a blue solution, may be found in normal urine undergoing decomposition, in the urine in peritonitis, pyelo-nephritis, etc. One also sees bundles of violet-red crystals or plates of indigo-red.

Melanin as amorphous scales has been found rarely in the sediment.

Hemoglobin in amorphous scales, plates, or casts occurs in the urine sediment in cases of hemoglobinuria.

Cholesterol.—Cholesterol, as flat superimposed plates often with re-entrant angles (see Fig. 22), is sometimes present in the urine in such amounts as to justify the term "cholesterinuria." It is found, always with other fats also, where large numbers of pus cells are undergoing fatty degeneration and when tissue is breaking down, therefore in some cases of vesical catarrh, in pyelitis especially, in pyonephrosis, echinococcus cysts of the kidney and sometimes in nephritis. It is rarely found in the tissue of kidneys which have undergone fatty degeneration and never in chyluria, in which condition especially one would expect to find it. Hirschlaff[148] determined even 5.8 gms. of cholesterol per 100 c.c. of urine in the urine of a case of hydronephrosis while the sac was emptying. We followed for some time a marked case of cholesterinuria of long standing in a case of renal cyst of doubtful nature.

Cholesterol is insoluble in cold, but easily in hot, alcohol, the precipitate reappearing as the alcohol cools. It is soluble in chloroform. If a solution of cholesterol be superimposed on concentrated H_2SO_4 its color becomes first blood-red and then more violet-red, while that of the sulphuric acid becomes dark red with a green fluorescence (Salkowski). This play of colors can be watched under the microscope if cholesterol crystals be brought into contact with H_2SO_4 4 parts and H_2O 1 part.

Leucin and Tyrosin.—Leucin and tyrosin appear in the urine in certain pathological conditions. Leucin is never a spontaneous sediment while tyrosin needles have been reported as a sediment in 3 cases, 1 of which was of acute yellow atrophy of the liver, and 1 of phosphorus poisoning. These substances in solution are often present in the urine of cases with acute yellow atrophy, phosphorus poisoning, smallpox (rarely), severe typhoid fever, pernicious anemia, leukemia and sometimes in cases with simple cardiac liver.[149] To demonstrate them it is as a rule necessary to evaporate the urine to about $\frac{1}{10}$ its volume and then add alcohol which will precipitate the needles of tyrosin and the spheres of leucin (as well as peptone and lactic acid). Tyrosin crystallized out in black needles grouped together like sheaves of wheat (see Fig. 48, *e*). Since patients with tyrosin in the urine are usually jaundiced one may confuse tyrosin with the intense brown needles of bilirubin which often have a rather similar shape. In an alkaline urine the calcium phosphate needles must be excluded. Leucin should be searched for in fresh urine since it may form rapidly in any decomposing albuminous urine. If pure it would appear in groups of spherules (see Fig. 48, *c*) which have little refractility, a much clearer contour than the spherules of the urates, no spicules, and a hyaline or a radiating structure. But as a sediment it is practically always impure and in dark spheres or masses or as spherules which may have a dark center and a clear periphery, or vice versa.

The presence of leucin in a sediment should always be confirmed by chemical tests.

To isolate tyrosin and leucin from urine it is necessary, first, to remove all albumin by heat and acid. The filtrate is first precipitated with neutral, then with basic, lead acetate until all precipitation ceases. The urine is then filtered, the lead in the filtrate removed with H_2S and the filtrate concentrated by evaporation. The tyrosin may begin even now to separate out slowly if present in considerable amount. The fluid should be concentrated to very small volume and the urea extracted by absolute alcohol. The residue is then boiled with weak ammoniacal alcohol and the filtrate is again evaporated to small volume and then allowed to crystallize. The leucin and tyrosin will separate out, that one first the solution of which first reaches saturation. A partial separation may now be accomplished by adding a small volume of alcohol which will dissolve the leucin more easily than the tyrosin. If after the above procedures no precipitate has appeared the residue and fluid are again diluted, precipitated with basic lead acetate and the process repeated.

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[148] Deutsches Arch. f. klin. Med., 1899, vol. lxii, p. 531.
[149] Mann, Quart. Jour. Med., October, 1907.

The leucin and tyrosin could be more completely separated by dissolving the residue after evaporation in boiling water plus a little ammonia and adding to the hot solution basic lead acetate, stirring all the while, until the precipitate is no longer brown but white. This is then filtered, heated nearly to boiling, made slightly acid with dilute H_2SO_4, and then boiled to drive off the ammonia and to precipitate the lead. It is then rapidly filtered and cooled. The tyrosin will now precipitate out almost quantitatively. The solution remaining is now heated with H_2S to precipitate the lead and then evaporated to smaller volume. While it is boiling freshly precipitated $Cu(OH)_2$ is added in excess and the boiling continued for a few minutes. The precipitate, which will contain part of the leucin, is suspended in boiling water, decomposed with H_2S, a little acetic acid added and then filtered. The filtrate is decolorized with animal charcoal and evaporated to small volume. On cooling, some of the leucin will now separate out and the rest will remain a blue copper compound. It is very difficult to obtain leucin pure although this can be done by forming its ethyl ester.

Tyrosin ($C_6H_4CH_2CHNH_2COOH$).—Tyrosin (see Fig. 48, *e*) will crystallize out from aqueous solutions in bundles of needles arranged like sheaves of wheat and from ammoniacal alcohol solution in bunches of prisms. These crystals are soluble in water, slightly so in alcohol, not at all in ether and are easily in acids and alkalies. Tyrosin cannot be positively identified from the appearance of its crystals. Chemical identification is necessary, yet these tests cannot be applied directly to a urine or to a mixed sediment. The sediment should be filtered out, washed with water, dissolved in ammonia plus a little ammonium carbonate in warm solution and then evaporated until it recrystallizes.

Piria's Test.—To some dry tyrosin in a test-tube are added a few drops of concentrated H_2SO_4, this at first warmed gently and then boiled in a water-bath for ½ hour. A red solution of tyrosin sulphate is formed. This is now cooled, several volumes of water added, is then neutralized with $BaCO_3$ and filtered. The filtrate is evaporated to a few cubic centimeters and weak, acid-free Fe_2Cl_6 added to the cooled solution. A fine violet color results. This test will be interferred with by any free mineral acids or by an excess of Fe_2Cl_6.

The hot aqueous solution of tyrosin gives with Millon's reagent, $(Hg(NO_3)_2+KNO_2)$, a fine red color and an abundant red precipitate.

Leucin $[(CH_3)_2CHCH_2CHNH_2COOH]$.—Leucin will separate out from a solution as spherules whose color and regularity of outline will depend on the purity of the specimen. The spherules often separate out in groups and frequently show a striation. Leucin (see Fig. 48, *c*) is soluble in water, less so in alcohol, but very easily in acids and alkalies. All of these compounds are more soluble in an impure than in a pure condition. No leucin is ever found in a urine sediment before the urine has been much concentrated. The methods for its isolation have been mentioned above. Before the chemical tests are applied leucin must first be purified by recrystallizing it from hot ammoniacal alcohol. Its characteristic tests are: Its crystal form when pure, the fact that it sublimes to a woolly mass at a gentle heat at 170° C. with fusion and with the odor of amylamine, Scherer's test and Salkowski's test.

Scherer's Test.—Pure leucin together with a little HNO_3 is evaporated on a platinum foil. A colored residue is obtained. This is warmed with NaOH and a water-clear if pure, or colored if impure, fluid results. If this is evaporated carefully an oily fluid is obtained which rolls around without wetting the foil. This test is characteristic for even impure leucin.

Salkowski's Test.—To the specimen is added a little water plus 1 or 2 drops of 10% $CuSO_4$. A blue solution is obtained, $(C_6H_{12}NO_2)_2Cu$, which does not reduce on heating.

Cystin.—Cystin, supposed to be a product of intermediate proteid metabolism which because of some perversion of metabolism not yet well understood escapes further cleavage, may appear for years, even for the individual's entire life, in the urine in large amounts. Cystinuria itself gives rise to no symptoms but the calculi it forms make the lives of their victims miserable and lead to repeated operations. Fortunately the formation of calculi is intermittent, allowing the patients long periods of relief while in some cases the removal of the stones would seem to be followed by the cessation of the cystinuria. The output of cystin in some cases is said to be intermittent.

Cystin is a normal intermediate product of proteid metabolism and contains the most, possibly all, of the sulphur of the proteid molecule. It is not a constituent of normal urine. If a normal person ingests cystin he will eliminate about 66% of its sulphur as inorganic sulphates and about ⅓ as neutral sulphur, but none as cystin. Simon and Campbell[150] think that some is eliminated in the bile as taurochloric acid. Why it should be excreted is not known. The theory that it is the product of an intestinal mycosis, which theory is borne out by the presence in both the intestinal contents and urine of certain diamines, as cadaverin, putrescin and others, is no longer held. Most believe that it is an individual variation in metabolism, a congenital inability on the part of the organism to oxidize the cystin nucleus which may be present in several of the same family.[151]

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[150] Johns Hopkins Hosp. Bull., 1904.

In the fresh urine of a case of cystinuria may be seen the transparent hexagonal crystals of cystin. These crystals (see Fig. 49) are quite characteristic, yet not absolutely so since uric acid may assume this exact form. Sometimes these crystals are massed into large concretions from a pin-head to 1 cm. in diameter, of a whitish-yellow color, rather soft and waxy and crystalline on cross-section. These crystals are soluble in ammonia and reprecipitated by acetic acid, a test necessary to exclude uric acid. We have seen but 4 cases. One has had many of these concretions removed by crushing. Another case, a woman, was distressed for years by these concretions but refused operation. As she has since attained considerable success in public life we presume that the concretions no longer bother her much.

The urine in such cases often gives on standing the odor of H_2S. It is in this condition particularly that the neutral sulphur of the urine is greatly increased. The neutral sulphur is indeed the best index we have of the amount of cystin present.

Diamines.—The presence of traces of diamines in the urine and in the feces has attracted some attention.[152] These found are putrescin sometimes, cadaverin sometimes, sometimes both. Their presence is variable and intermittent. Lewis and Simon state in 1902 that they had been found in 7 cases.

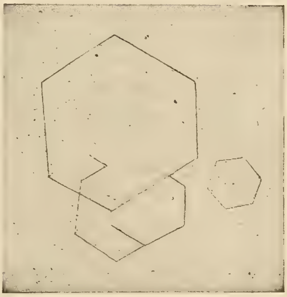

FIG. 49.—Cystin crystals from urine. × 400.

Baumann's method for their detection is as follows: The 24 hour amount of urine is shaken up with 10% NaOH and benzoylchloride (in the proportion of 1500 : 200 : 25) until the odor of benzoylchloride is gone.

The precipitate (of phosphates, carbohydrates, and benzoylated diamines) is filtered with the aid of a suction-pump. The precipitate is digested with alcohol, filtered, the extract evaporated to small volume, 30 volumes of water added and then allowed to stand for from 12 to 48 hours. The benzoylated diamines will now separate out in the milky fluid as a voluminous sediment of white crystals. This is redissolved in alcohol, concentrated to small volume and diluted again with water. This process is repeated several times to separate the carbohydrates.

More of the diamines may be recovered from the first filtrate by acidifying it with H_2SO_4 and extracting it 3 times with ether. To the ether residue 12% NaOH is added till the fluid is neutral, then 3 to 4 volumes of the alkali. If this is then kept in a cold place cystin and the diamines will separate out. They are filtered out and suspended in cold water; the benzoylchloride crystals remain.

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[151] Kretschmer, The Urologic and Cutaneous Review, 1916, xx, No. 1.

[152] Simon, Am. Jour. Med. Sci., 1900, vol. cxix, p. 39; 1902, vol. cxxiii, p. 838; Schollberg and Garrod, Lancet, August 24, 1901.

The crystals are dissolved in a little warm alcohol and 20 volumes of ether added. Benzoylputrescin will be precipitated. Its melting point is from 175° to 176° C. The ether residue contains benzoylcadaverin the melting point of which is 129° to 130° C.

Unorganized Sediments.—The following outline given by Neubauer and Vogel for recognizing an unorganized sediment is so useful that we quote it in full.

A. Acid urine.
  (*a*) Sediment amorphous.
    (1) Sediment consists of fine granules in clumps, mingled with which are crystals of uric acid and of calcium oxalate; urate sediment. This sediment is soluble on warming, and if a drop of strong acetic acid be added the granules gradually disappear with the subsequent separation in a few hours of uric acid crystals.
    (2) Dumb-bell shaped bodies.
      (*a*′) Insoluble in strong acetic acid and soluble in concentrated hydrochloric acid without subsequent crystallization; calcium oxalate.
      (*b*′) Insoluble in concentrate hydrochloric acid: probably calcium sulphate. This sediment should be filtered, washed, dissolved in much hot water and tested for calcium and sulphuric acid separately.
    (3) Very refractive globules, soluble in ether: fat.
    (4) Amorphous yellow granular masses: bilirubin or hematoidin.
  (*b*) Sediment crystalline.
    (1) Yellow or brown whetstone-shaped crystals, single or in rosettes; alone, or with amorphous urates and calcium oxalate: uric acid. These crystals are soluble in sodium hydroxide and reprecipitated by an excess of concentrated hydrochloric acid.
    (2) Small yellow rhombic tablets alone or with amorphous granular tablets of the same color, often embedded in tissue detritus: bilirubin or hematoidin.
    (3) Colorless (or yellow in a decomposed urine), transparent, strongly refractive octahedrons, double envelope forms, or quadrangular short narrow prisms with octahedrons at the ends, insoluble in acetic acid and soluble in hydrochloric acid: calcium oxalate.
    (4) Crystals somewhat similar to the last mentioned, or large coffin-lid crystals, soluble in acetic acid: ammonium magnesium phosphate (triple phosphates).
    (5) Symmetrical hexagonal tablets, sides and angle almost equal, insoluble in acetic acid, soluble in ammonia: cystin.
    (6) Colorless whetstone-shaped tablets, insoluble in acetic acid; soluble in ammonia. On the addition of hydrochloric acid to this sediment in solution hexagonal tablets separate: xanthin.
    (7) Large, flat, strongly refractive elongated rhombic tablets, soluble in acetic acid, and partially so in ammonium carbonate: normal magnesium phosphate.

- (8) Prisms, single or in rosettes.
  - (*a*′) Soluble in ammonia: hippuric acid.
  - (*b*′) Insoluble in ammonia and in acids: calcium sulphate.
- (9) Wedge-shaped prisms, single, or in clusters or thick rosettes, decomposed by ammonium carbonate and soluble in acetic acid: monacid calcium phosphate.
- (10) Bunches of very fine needles insoluble in acetic acid and soluble in ammonia and hydrochloric acid: tyrosin.

B. The urine alkaline when the crystal precipitates (after the urine becomes alkaline many of the sediments previously mentioned which separated in the acid urine may still remain).

- (*a*) Amorphous.
  - (1) Small granules together with triple phosphate crystals.
    - (*a*′) Soluble in acetic acid without gas formation: normal phosphates of the alkaline earths.
    - (*b*′) Soluble, but with gas formation: carbonates of the alkaline earths.
  - (2) Dumb-bell shaped masses or large spheres, soluble in acetic acid with gas formation: calcium carbonate.
  - (3) Large dark spheres often covered by small projecting crystals: ammonium urate, soluble in hydrochloric acid or acetic acid with the subsequent separation of the rhombic crystals of uric acid.
- (*b*) Crystalline.
  - (1) Large colorless prisms, many coffin-lid in shape: triple phosphates, easily soluble in acetic acid.
  - (2) Rosettes of very fine blue needles or blue tablets: indigo.
  - (3) Rosettes of violet-red needles or rhombic platelets: indigo-red.

Chyluria.—Chyluria is a condition characterized by the presence in the urine of enough fat in emulsion to give the urine a milky appearance. When the amount of fat is sufficient to give only an opalescent appearance the term lipuria is used, although the latter term should include both.

There are 2 forms of chyluria, the 1 due to filaria infection, and the non-parasitic form, the etiology of which is not understood. Some claim that in the latter cases the source of the fat cannot be lymph as it is in the parasitic form since the urine contains no sugar as does lymph and also since there is often a higher percentage of fat in the urine than in lymph. Again, there is no decrease in the percentage of the normal urine constituents as would be the case were the urine diluted by another fluid.

In chyluria the fat may form gross tallow-like masses, but as a rule the droplets are even much finer than those of milk. The urine may resemble milk, in other cases whey, but often it has a reddish tinge due to blood. The fresh urine is weakly acid or neutral in reaction and does not have the usual urinary odor. On standing a cream sometimes rises and a fibrin coagulum often forms. In addition to fat such urine always contains serum globulin and serum albumin and sometimes cholestrol and lecithin. Fibrinogen has been found, also hemialbumose and peptone. The amount of proteid present varies from 0.2 to 2% or more and the fat from a trace to 3%. A few leucocytes and a few red blood-cells may be found. In both forms urinary casts, etc., are absent unless Bright's disease also is present. The urine may be chylous during the night and clear during the day, or vice versa. In other cases the excretion of the fat is intermittent, appearing only when the patient is in a vertical position, after digestion, after bodily exercise, or after excitement, etc. In some cases coagula form in the bladder and cause considerable trouble. In parasitic chyluria one finds also in the urine, usually in the coagula, the eggs and embryos of filaria. Parasitic chyluria is a disease which lasts, often with intermissions, from months to years. It may cease spontaneously. This disease is endemic in certain tropical and subtropical regions, while a few cases are met with in the temperate zone. In these cases all the fat would seem to come directly from the pelvic lymphatics.

In some cases a fat diet increases a chyluria and a foreign fat may even be recognized in the urine. Chyluria would seem not to be due to a renal lesion. Claude Bernhard held that it was due to hyperlipemia and this to the inability of the body to burn fat; but an hyperlipemia is rare and this alone would not explain the albuminuria also present. Others ascribe chyluria to liver disease. Franz and Styskal believe that the fat comes directly from the lymphatic vessels since the chyluria will diminish or disappear if the patient be fed a fat-free diet or is starved, also since foreign fats of the food can be identified in the urine; and, finally, since the cells in the urine are lymphocytes.

Lipuria.—Lipuria differs from chyluria chiefly since the urine is opalescent rather than definitely milky. Lipuria is often mentioned in the urine reports in hospitals, but by beginners who have not yet learned to exclude the oil in the catheterized specimens of urine. The gross or microscopical appearances, however, are not sufficient for the recognition of fat. The urine should be extracted with ether and the residue examined chemically. Fat when heated gives off the odor of acrolein; the residue will make a fat-spot on paper, and will give the osmic acid test.

The student should exclude deception, the fat used during catheterization, that from the rectum, the tenacious phosphate sediment and the scum of bacteria which forms at the surface of the urine. Under normal conditions there is little if any fat in the urine. Lipuria is said to result from the over-ingestion of fat as a food or as a medicine (*e.g.*, cod-liver oil), the so-called "alimentary lipuria"; from the subcutaneous injection of oil, or an excess of oil rubbed into the skin. Lipuria is said to follow crushing or tearing of the subcutaneous fat, injury to the liver, or to fatty tumors, fracture of the bones, especially if the marrow be crushed and, rarely, acute osteomyelitis. It is said to develop in eclampsia, which disease formerly was supposed to be due to the crushing of the fat in the pelvis of the kidney. Lipuria has been reported in diabetes mellitus, alcoholism, tuberculosis, adiposity, nephritis, certain mental diseases, pancreatic diseases, cardiac diseases and after various protoplasmic poisons. A lipemia has been proved coincident with fractured bones, subcutaneous bruises, and in diabetes mellitus. The claims for other diseases should be confirmed. The slight lipuria seen in nephritis in various infections, intoxications, anemias and cachexias may be due to fatty degeneration of the kidneys. The fat may arise also from epithelial cells, leucocytes, casts and fragments of tumors which have undergone fatty degeneration, but under these conditions the most of the fat remains in the cells or collects in droplets which float on the surface.

ORGANIZED SEDIMENTS

Mucous Sediment.—The "nubecula" is a very faint cloud of mucous strands which appear in the top layers of the urine soon after it cools and later sinks to the bottom of the glass. This mucus is from the epithelial cells of the urinary passages. The mucous strands enclose a few "mucous corpuscles," *i.e.*, epithelial cells and mononuclear or polymorphonuclear leucocytes, some ameboid in the fresh urine, and some crystals. If because of "catarrh" of the urinary passages a good deal of mucus is present it may form a definite translucent or cloudy coagulum-like sediment better seen after the addition of a little acetic acid.

Epithelial Cells.—Renal Epithelial Cells.—The epithelial cells from the kidney tubules (see Fig. 52, *e*) are round or cubical in shape and larger than leucocytes (12 to 25μ) from which they are easily distinguished by their large vesicular nucleus. Their protoplasm is nearly always fatty, either finely so or so very fatty that they may resemble colostrum corpuscles (c. h.). These cells sometimes show a definite myelin degeneration similar to that of the alveolar epithelium cells of the sputum (see Fig. 52, *d*).

It is claimed that renal epithelial cells are found in normal urine but the chances are that the majority of cells thus reported are endothelial leucocytes. Renal cells appear in the urine in all forms of nephritis, but especially in the subacute parenchymatous variety in which disease one finds them single, in clumps, or attached to casts. In cases of renal infection they may be found in the masses of pus-cells (see Fig. 52).

Hemosiderin in the Urine.[153]—To demonstrate hemosiderin in the urine a fresh specimen preferably warm from the body, is centrifugated at high speed and the supernatant fluid poured off as completely as possible. The sediment is suspended in the trace of fluid that remains and slide preparations studied microscopically for suggestive orange or brown granules, more particularly in the cells. A mechanical stage should be used and 10 minutes at least given to the search. As a rule the sediment from a 20 c.c. specimen will yield a fair number of cells from the higher portions of the urinary tract, but often that from 60 to 100 c.c. must be obtained. In urine allowed to cool prior to centrifugation the nubecula may prevent proper concentration of the formed elements. Kept urines which have become cloudy with urates may be cleared by warming. To search a heavy crystalline sediment, or one poor in cells, or containing only leucocytes and squamous epithelium is time wasted. Female patients should be catheterized since hemosiderin may come into the urine from the genital tract.

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[153] Rous, Jour. of Exp. Med., 1918, vol. 28, p. 645.

For the Nishimura test the fresh sediment, as free as possible from urine, is mixed with a little human serum untinted with hemoglobin and thick films are made and dried. These are fixed by heat, placed in strong ammonium sulphide for 1 hour, washed briefly with water and subjected for 20 minutes to a fresh mixture of 2% potassium ferrocyanide and 1% hydrochloric acid in equal parts. After another brief rinsing with water the preparations are stained in lithium carmine for a few minutes, differentiated in acid alcohol (1% HCl), and run rapidly through 95% alcohol, absolute alcohol, xylol and mounted in balsam. The acid alcohol differentiates the red of the carmine and turns the iron granules a deep blue. Its action should be carefully followed with the microscope since if prolonged it will dissolve the iron, or at least cause the blue tint of the latter to run and fade.

By this method permanent mounts are obtained to be looked over at leisure. The iron granules stand out in deep blue against the general carmine tint.

The presence of cells in the urine containing hemosiderin (the free granules are to be disregarded since they may have a different origin) indicates merely the presence of an actual siderosis of the kidney parenchyma. Their presence would be important in the diagnosis of hemachromatosis in cases of doubtful skin pigmentation. These cells are present in hemolytic jaundice after a siderosis of the kidney has developed while their presence would in a doubtful case speak in favor of pernicious anemia.

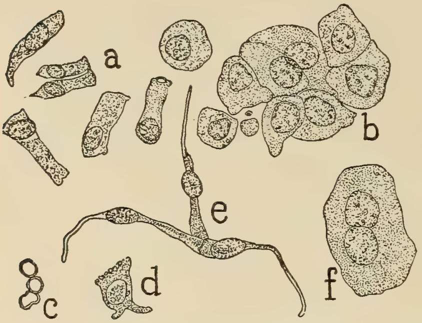

FIG. 50.—*a, e, d,* cells from male urethra; *b, f,* cells from transitional epithelium; *c,* shadows of red blood-cells. × 400.

EPITHELIAL CELLS FROM THE URINARY PASSAGES (see Fig. 51, *b, c, d,* and Fig. 50, *b, f*).—A few *surface cells of the transitional epithelium* of the urinary passages are present in the nubecula of normal urines. These are increased in number and in variety in inflammatory and destructive lesions of this mucosa by the appearance of cells from the middle and lower layers of this epithelium. The flat, polygonal, squamous epithelial cells (see Fig. 51, *e*) from the prepuce, end of the ureter, vagina or fossa navicularis cannot always be distinguished from the superficial cells from the bladder, although usually the stratified grouping of those from the vagina makes their recognition easy.

The cylindrical cells (see Fig. 50, *a, e, d*) of the urethra are long, narrow and bluntly pointed. They occur in pairs or clusters.

The cells from the transitional epithelium of the urinary passages differ in appearance according to the layer from which they originate. Some are large and irregular, others round or polygonal. The former are flat, with clear protoplasm and usually with a small, very distinct central nucleus. Their edges are sometimes very refractile, thin and horny. These are the typical pavement cells from the superficial layers of transitional epithelium. They are found in large numbers in the urine of patients who are irrigating their bladders with too strong fluids, in which cases they may be desquamated in large sheets. Dawson[154] who studied such a case found that they varied greatly in size and shape. Some were irregular, large and polygonal, some smaller and hexagonal. The larger often had a peripheral non-granular zone. The nuclei were round or oval, sharply defined and central and in many cells were budding. Among these cells were large giant-cells with even 15 nuclei. In no cells did he see the cupping of the under surface so often described.

The smaller polygonal or elliptical cells from the deeper layers of the mucosa have a very granular protoplasm and a large nucleus (Fig. 51, *a*, *b*, *d*). Others have a cell body which is definitely oval, or conical, or even threadlike, some with 2, 3 or more branches and a very distinct nucleus.

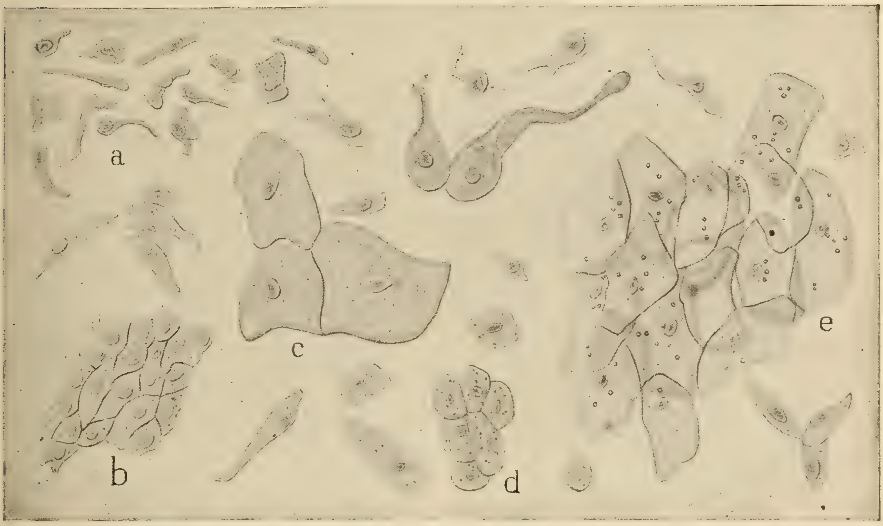

FIG. 51.—Various forms of epithelium cells in the urine: *a*, "tailed" cells; *b*, *d*, small polygonal; *c*, large surface cells; to the right of *d* is a small round cell of uncertain origin; *e*, squamous cells from vagina. All of these cells except *e* were obtained by ureteral catheterization, hence from the pelvis of the kidney or scraped from the mucosa of the ureter. The latter is especially true of *b*, *c*, *d*, and neighboring cells, which are the forms one gets from normal cases; *a*, were from cases of pyelitis. × 400.

These, described as spindle cells or "tailed" cells, were believed formerly to come from the pelvis of the kidney. Finally, some from the deeper layers of the mucosa are small, round, with a round nucleus and resemble mononuclear leucocytes (see Fig. 51), which, indeed, some of them may be. The best urine in which to study these epithelial cells is that obtained by ureteral catheterization. Such cells may arise anywhere along the transitional epithelium from the pelvis of the kidney to the bladder, singly or in clusters.

The claim that one can tell from the appearance of single cells where in the urinary tract they came from is easily disproved. Classes in clinical diagnosis should study macerated specimens of this transitional epithelium scraped at various points along the urinary tract. Sahli considered that a predominance of tailed cells over other epithelial cells would suggest a pyelitis. We agree with this.

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[154] Johns Hopkins Hosp. Bull., July, 1898, p. 155.

In a case of streptococcus pyelitis the urine obtained at autopsy from the pelvis of the kidney contained great numbers of small round polygonal and tailed epithelial cells in groups, scores in each field (of 400 magnification) and 3 to 4 of the large polygonal cells in each field. Pus-cells were present in great numbers; very little mucus was seen.

The smaller polygonal cells in groups (Fig. 51, *b*, *d*) predominate in urine obtained through ureteral catheters.

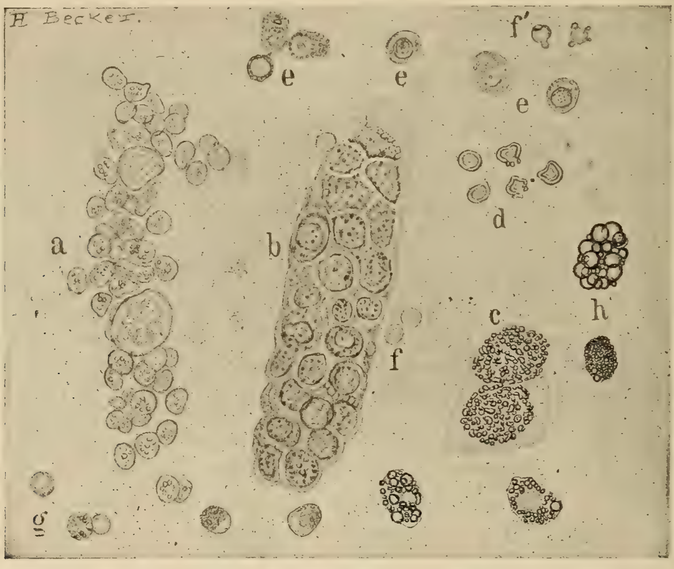

FIG. 52.—*a*, pseudo pus-cast; *b*, epithelial cast showing protoplasmic bridges between cells; *c*, two very granular (myelin ?) renal cells; *d*, myelin globules; *e*, renal epithelial cells; *f*, crenated red blood-cells; *g*, pus-cells; *h*, very fatty renal epithelial cells. × 400.

Casts.—Casts are cylindrical masses of coagulated hyaline or granular material moulded into a fairly solid mass, formed in the lumen of the tubules and washed out by the urine. They have been classified as: cellular, granular and amorphous, but the most are combinations of these.

EPITHELIAL CASTS (Figs. 52 and 55).—The epithelial casts are made up in part at least of renal epithelial cells. Some are aggregations of desquamated cells massed together, with one cell at least well enough preserved to be recognized as a renal epithelial cell; others are actually fragments of tubules, with lumen preserved and the cells so intact that even the intercellular protoplasmic bridges are visible (Fig. 52). These are the so-called "epithelial tubes." In sections of the renal cortex invaginated portions of tubules can be seen which, broken off, would be just such casts. The cells of epithelial casts may be well preserved, or present all grades of fatty or granular degeneration. All transitions between these and the coarsely granular and fatty casts are seen and, indeed, the most of these would be called epithelial could one cell be recognized. If a cast which is definitely of some other type, as hyaline, has even one renal epithelial cell attached it would be an epithelial cast since it would have the significance of one.

Granular Casts (Fig. 53).—The granules of granular casts may be coarse or fine. Of the fine we shall speak later. Coarsely granular casts may have the same size and appearance as epithelial casts except no cell is well enough preserved to allow its recognition. Some may originally have been pus casts. Others are cylinders composed of coarse granules with nothing in their appearance suggesting cells. The coarsely granular casts have a dense, opaque appearance and dark yellowish color. The most of the granules are soluble in acetic acid but usually a few fat granules are present. The most of these casts probably are made up of the detritus of epithelial (or pus) cells which underwent disintegration before or during the formation of the cast. (We have no direct proof of the transformation of one type of cast to another after the cast has left the kidney.) Hemoglobin casts which consist of masses of brownish-red pigment are often grouped as coarsely granular casts.

Fig. 53.—Coarsely and finely granular casts. × 400.

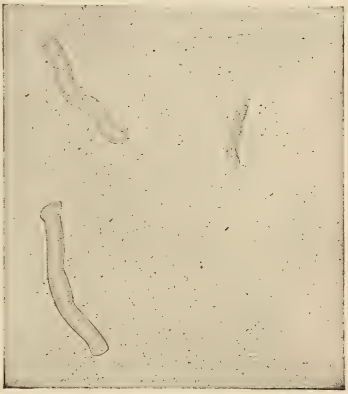

Fig. 54.—Waxy casts. × 400.

The finely granular casts present a very different problem. They are rather pale and translucent in appearance and seem composed of a finely granular material. None of these fine granules are of fat. These seem more closely related to the hyaline than to the coarsely granular casts and some are hyalines with a few fine granules attached. It would seem that the formation of these casts does not necessarily involve the actual destruction of entire renal cells, as would seem to be the case of epithelial and coarsely granular casts, but rather of their edges lining the lumen of the tubules.

Fatty Casts (see Fig. 55).—These striking objects are cylinders made up of fatty globules often in clusters which suggest the outlines of the original epithelial cells. The granules are yellowish or even blackish in appearance and are soluble in ether. Fatty acid crystals project from some.

Waxy Casts (see Fig. 54).—These casts are composed of a very refractive, clear, homogeneous material suggesting wax. They have sharp contours, are often of a white or yellowish color and show a great tendency to split transversely as though very brittle. These may be the longest of all casts and extend across the field with twists resembling a corkscrew, or the shortest and these suggest fragments of longer casts. Some give the amyloid reaction, others do not. They are not characteristic of amyloid degeneration of the kidney as was formerly supposed and yet in the urine of a recent case of amyloid disease it happened that practically every cast seen was a waxy cast. In general there are 2 very distinct forms of these casts—the yellowish and the bluish. The former, which resemble beeswax, were formerly called fibrin casts; the latter resemble paraffin. Waxy casts may be found in the urine of any case of nephritis in which granular casts occur and are especially numerous in the scanty urine passed while water retention is marked and especially just before death.

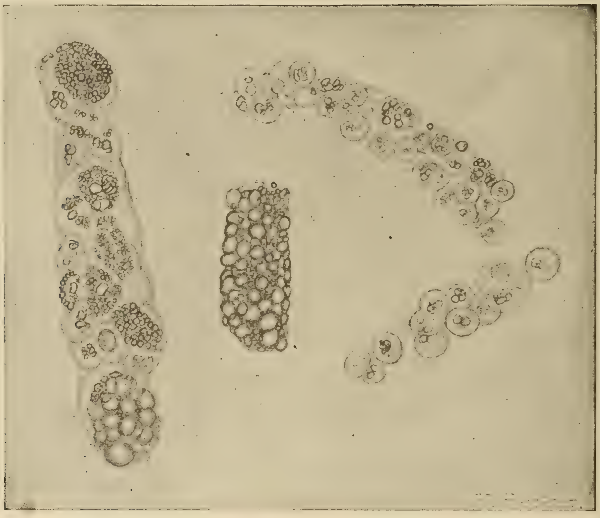

Fig. 55.—To the left an epithelial cast with very fatty cells; in the center a fatty cast; to the right two leucocyte casts. × 400.

In urine secreted just before death one may see most beautiful waxy casts. On one such case the granular and waxy casts were numerous but no hyalines were seen. These casts were enormous, many granulars measuring 0.136 mm. and the waxy 0.102 mm. in diameter. The latter looked as if made of paraffin. In another case, however, no waxy casts were found, only hyalines; yet these were not typical, since too refractile, yet they were not waxy. In other such cases all forms were found.

In addition to the definitely waxy and hyaline casts are many which have not quite the refractility of the former nor do they give the same color tests, and yet are more refractile than the hyalines with which they are classified. One finds many in some antemortem specimens. It is hard not to believe that they are transitional forms.

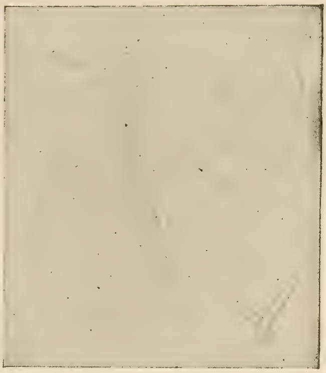

FIG. 56.—Hyaline casts of urine. × 400.

HYALINE, COLLOID OR GLASSY CASTS (see Fig. 56).—The most common form of casts, the hyalines, are so colorless, so translucent that they are easily overlooked unless the light is almost shut off, or unless crystals or cells are attached to them. For this reason some as a routine stain urinary sediments with Lugol's solution or with analine violet. Their outline is very regular, they give the microchemical tests for albumin and are soluble in acetic acid. They may have the same cells attached to them as have all the above-mentioned casts in which case the cells give them their name.

BLOOD-CASTS (see Fig. 57).—Blood-casts are either true coagula of red blood-cells which have formed within the tubules, or one of the above-described casts with at least 1 red blood-cell attached. Some of the blood-cells are so pale that it is hard to recognize them.

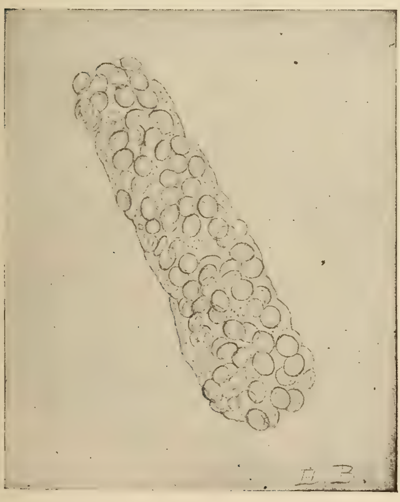

FIG. 57.—Blood-cast. × 400.

HEMOGLOBIN CASTS.—Casts composed of amorphous masses of hemoglobin are seen in hemoglobinuria. Others seem to be hyalines or granular casts impregnated with hemoglobin.

PUS-CASTS (see Fig. 55) are similar in the method of their formation to the blood-casts. The pus-cells appear more spherical than the epithelial but to be certain of their nature one should make their nuclei evident by adding acetic acid. The most of the pus-casts are hyaline or granular casts with at least 1 leucocyte attached. Those which are conglomerates of pus-cells are rare.

CYLINDROIDS (see Fig. 58).—The threads of mucus in the urine are either the so-called "mucous threads," which are flat ribbons of mucus which do not at all resemble hyaline casts, which often extend over several fields and vary much in diameter along their course (such threads make up the nubecula) and the so-called cylindroids which may closely resemble casts. These differ in appearance from hyalines in that one or both ends taper off into a longer or shorter thread. These have not the fibrillar appearance of mucous threads and chemically they resemble casts. Since they occur where true casts would be expected some claim that they have the same significance as they. The problem is a difficult one but we call no structure a true cast until we are sure that neither end runs off into a thread. Cylindroids covered by urates have the appearance of granular casts. The point is an important one, for if they are mucous threads they certainly arise from the mucous surface, while if casts they should arise in the renal parenchyma. True mucous threads arise in the bladder chiefly. One seldom sees them in urine catheterized from the pelvis of the kidney. They are insoluble in acetic acid while many cylindroids are soluble.

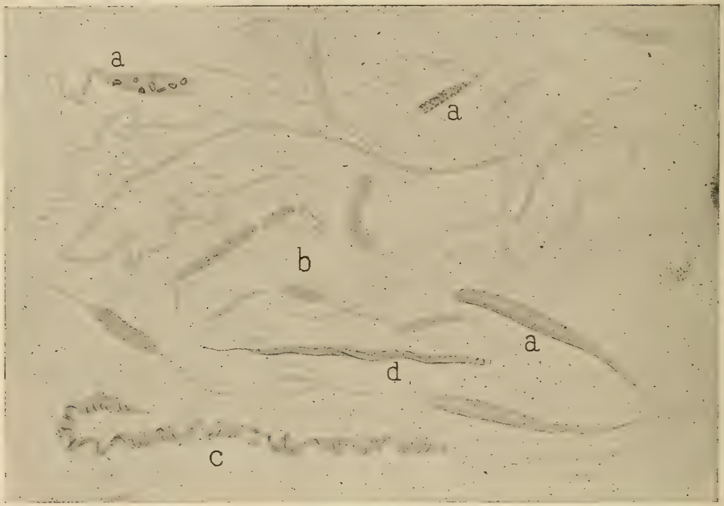

FIG. 58.—*a*, cylindroids, *i.e.*, bodies much resembling hyaline casts; *b*, mucous threads; *c*, a spiral structure of material resembling hyaline casts or mucous threads; *d*, a vegetable thread. × 400.

COMBINED CASTS.—A cast may be clear, therefore waxy or hyaline, at one end and granular at the other; or it may have cellular elements attached. All combined casts take their name from the cells attached and if a variety of cells are involved, from that one which would be most important in prognosis.

BACTERIAL CASTS.—Bacterial casts are masses of bacteria in the shape of a cast which would seem to be moulds of the tubules. These occur in purulent infectious pyelonephritis and in pyemic kidneys. Other casts may become permeated by bacteria in a remarkably short time.

URATE CASTS.—In the urine of the new-born with uric acid infarcts of the kidney may be found casts which are masses of sodium urate.

Pseudo-casts made up of urates are common. Other casts in a concentrated urine may become incrusted with urates and hence be more dark, homogeneous and granular then true granular casts. The urate masses also have uneven edges and disappear on warming. Scratches in the glass are sometimes confusing (Fig. 59). In some cases of pyuria (cystitis, *e.g.*) the mucus threads full of pus-cells make very perfect pseudo-casts (Fig. 52).

The length of true casts varies from very small fragments to 1 mm. or longer. Some are narrow, others broad. From the size of the casts no conclusions can be drawn of their source so much does the size of the tubules vary in pathological conditions. Some can almost be seen with unaided eye. It was formerly supposed that the beautiful corkscrew forms so often seen come from the convoluted tubules, but this is improbable since any corkscrew shape would probably be effaced during their passage through the straight tubules. Some are spiral all their length, others only at one end. This, says Senator, merely shows that they are composed of a tough elastic material and have been squeezed through a narrow orifice. The end of the cast is seldom split or forked.

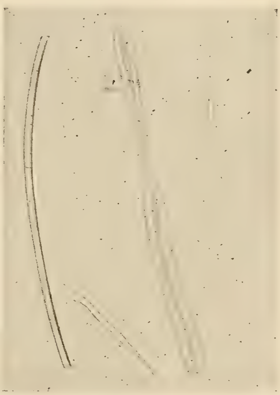

Fig. 59.—Pseudo-casts. From left to right, a linen thread, a vegetable spine, a cotton thread, and a scratch on the glass slide.

The *origin of epithelial casts*, especially of those with a lumen, is not disputed; nor is that of those blood- and pus-casts which are conglomerates of cells. The coarser granular casts quite certainly are made up from the detritus of epithelial or pus-cells. All transitions from the coarsely granular to the waxy casts may be found, especially in sections of kidneys. The origin of the hyaline casts, however, has long been in dispute. Some claim that they are a coagulated exudate from the blood into the tubules, others a product of the secretion of the epithelial cells themselves; that is, the slightly injured epithelial cell may furnish an abnormal secretion of coagulable material which coagulates in the tubules. Hyaline globules can be demonstrated in these cells and the confluence of these in the tubules would explain casts. This is the generally accepted idea. Another explanation, for which the study of microscopic sections of kidneys gives evidence, is that the fine edge of these epithelial cells undergoes hyaline degeneration, sloughs off and is moulded into a cast. Hyaline casts are certainly not composed of coagulated fibrin. (They arise where there is no suspicion of inflammation, as in a practically normal kidney, *e.g.*, the albuminuria of the new-born; they do not give the Weigert's fibrin stain.) They are not simply coagulable albumin, for where this is present, as in cases of chyluria, these casts may be absent. There is no evidence that the albumin of the blood is their source, since albumin and casts are little if at all related in their origin, for either one may be present without the other. There is evidence that if hyaline casts remain unusually long in the tubules they become waxy (see page 266).

The origin of hemoglobin casts is an interesting problem since hemoglobin is soluble in urine. It may be, however, that these are hyaline or waxy casts impregnated with hemoglobin.

The *chemistry of casts* has been but little studied. Probably none are composed of fibrin. Whether any consist of amyloid or not is disputed. Very few give a typical amyloid reaction, the majority of those which look waxy taking merely a brownish color with Lugol's solution and a reddish color with gentian violet. Most genuine casts are soluble in acetic acid, and this is a valuable aid in distinguishing them from mucous cylindroids.

DIAGNOSTIC IMPORTANCE OF CASTS.—It is claimed that casts sometimes appear in the urine of persons whose kidneys are quite normal. This is not generally admitted. The disturbance leading to the cylindruria may be but slight and transitory but their presence is always evidence of an abnormal condition of the renal epithelium. This may be a temporary disturbance of the renal circulation, a temporary condition of malnutrition, a mild irritation, congestion, pressure, etc.; or the cause may be permanent and serious, as in nephritis. It is quite true that from the number and character of the casts present in the urine one cannot judge as to the severity of the cause of the cylindruria. Indeed, it would seem that, the more normal the condition of the cells prior to the renal disturbance the more brilliant will be the display of casts. Certainly the most seriously injured (small, contracted) kidneys may for fairly long periods excrete urine containing few or none.

The general statement may be made that casts appear under the same circumstances and have the same general significance as albumin, although it would seem that of the two the albumin is a little more sensitive index of renal disturbance than are casts.

While albuminuria and cylindruria are usually associated, either may appear without the other. Casts have been reported absent when albumin was present in some cases of chronic nephritis (especially the arteriosclerotic type), in some cases of jaundice and in some of febrile albuminuria. To prove casts absent, however, is no easy matter since in some urines they disintegrate very rapidly. The search should be made with the aid of a centrifuge while the urine is very fresh. The rapid disappearance of casts has been ascribed to the presence in the urine of a ferment which some claim is pepsin, others, a bacterial enzyme.

On the other hand, it is not at all rare to find casts in urines in which albumin cannot be demonstrated by clinical methods. A pure cylindruria may be due to some food, as asparagus, radishes, coffee and mustard; or to a drug, as alcohol, salicylic acid, mercury, arsenic and camphor; or to an injection of tuberculin, etc. Pure cylindruria is sometimes seen in cases of heart disease, cancer, jaundice and constipation; in acute infectious diseases, as scarlet fever, typhoid fever, erysipelas and tuberculosis; in nephritis, especially chronic, in acute cases especially during convalescence and even in uremia. In fact, in any case in which albuminuria would be expected cylindruria alone may occur. It is of interest that among the athletes studied with reference to "physiological albuminuria" were several with pure cylindruria. In a recent case of brain tumor we found in the urine many epithelial and waxy casts and many cylindroids, but no albumin.

In many cases there is little doubt but that both casts and albumin were for a time present, but that the latter disappeared first. We believe that the casts are apt to disappear before the albumin in those cases in which the renal lesion is chiefly degenerative, as in patients with arteriosclerosis; the albumin first, in those cases in which the lesion is inflammatory, as in parenchymatous nephritis.[155] Without much doubt our failure to find more cases of pure cylindruria has been due to our habit of not examining the sediment carefully if the test for albumin is negative.

The association of casts and "nucleo-albumin" but no serum albumin is often noted (see page 222).

In any given case of nephritis there usually is a rough parallelism between the number of casts and the amount of albumin in the urine, but the casts are a much more variable factor than is the albumin. They are most abundant in acute and subacute parenchymatous nephritis, and fewest in interstitial nephritis, amyloid disease and chronic passive congestion. There is no evidence as yet for a special form of nephritis with cylindruria its most prominent urine feature. In the diagnosis of the grade of a case of renal trouble the number and variety of casts are not as important as are the specific gravity of the urine, its chemical analysis, the blood chemistry and especially the history and physical condition of the patient. Casts, together with albumin, would seem to depend more on the acute element of the process called nephritis than on the total lesion. There are no casts which are pathognomonic of nephritis; any or all varieties may appear in the urine of patients with nephritis and also of patients who have no true renal disease. But nephritis is practically the only condition in which cylindruria is long-continued; in other cases it lasts but a few hours or days.

"Showers" of casts, *i.e.*, the sudden appearance of numbers of casts greatly in excess of that of the preceding or of the succeeding days, usually last but part of a day. These may occur in any form of nephritis.

In the cylindruria not due to nephritis the casts are as a rule few and these usually are hyaline. But in the urine of athletes following great strain all varieties may be found (see page 225). Epithelial and leucocyte casts are not nearly so rare as is imagined and may occur in even non-inflammatory transitory cylindrurias.

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[155] Emerson, Jour. of A. M. A., Jan. 6 and 13, 1906; Vincent, N. Y. Med. Jour., April 13, 1907.

It was formerly supposed that the presence of epithelial and the hyaline casts meant an acute process, that of granular and waxy casts a more chronic process; but in all forms of nephritis all kinds of casts may appear; in amyloid disease even there is nothing characteristic in the urine picture. Sahli suggests that casts in the forming become granular and waxy casts from lying a long time in the tubules and that this explains the large numbers of these present in the urine after a period of suppression, as after an acute nephritis or an acute exacerbation of a chronic form. We have noticed this also in other oligurias; that, for instance, following decapsulation of the kidneys. During the first few days after the operation as the urine begins to increase in amount a very large number of waxy casts appear.

Almost any kind of casts may appear following various renal disturbance. Brown[156] has reported some interesting results of operation on normal kidneys (nephropexy or exploratory nephrotomy). On the first day after the operation the urine contained casts in enormous numbers, hyaline, granular and epithelial. Considerable albumin also was present. These casts rapidly diminished in number and in from 2 to 6 days entirely disappeared. During this time there were no symptoms of nephritis, no edema and no change in the amount of urine. The disproportion between the small amount of albumin and the great number of casts was a marked feature of these casts. There were no later symptoms.

Casts in great numbers are important as a prodromal symptom of diabetic coma (Külz). These may appear in immense numbers before the coma begins and even form a macroscopic sediment. These casts are characteristic in appearance—short, broad, of delicate outline, pale, the most of them granular and hyaline and with few other formed elements.[157]

Staining Casts.—All methods of staining casts are unsatisfactory, because the stain precipitates in the urine or the albumin of the urine may itself take the stain. The specimens cannot be dried for this reason. The best method is to wash the casts 1 or 2 times by sedimentation with 0.6% sodium chloride solution to rid them of all soluble matter and albumin. In the next centrifugalization 1% methylene blue may be added. To hasten centrifugalization a little alcohol should be added, not much, nor should it be allowed to remain for a long time in contact with the sediment else a coagulum will result.

To preserve the casts and also to stain them, they should be washed as above in normal salt solution and lastly in a 1% osmic acid solution, or in 1 to 10% formalin, or in a 5% $HgCl_2$ solution for 5 minutes. In the latter case they are then washed with water and preserved in from 2 to 10% (or 1 to 2%) formalin. If no red blood-cells are present the mercuric chloride should not be used since it disturbs microchemical tests. In case formalin is used the casts should be especially well washed or the spherical crystalline masses of diformaldehydurea will form. Gumprecht adds that it is not really necessary to wash the casts if they are well centrifugalized and the supernatant fluid completely decanted. A good staining method for fat and cell nuclei was described by Cohn.[158] The specimen, well washed by centrifugalization in normal salt solution, is air-dried on the cover-glass and hardened by immersing the glass in 10% formalin for 10 minutes. It is then washed rapidly but gently with H_2O and then immersed for 10 minutes in a concentrated Sudan III solution in 70% alcohol. It is then washed in 70% alcohol for 1 to 2 minutes and then stained briefly in hematoxylin (Ehrlich's solution). The specimens are mounted in glycerin.

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[156] Johns Hopkins Hosp. Bull., May, 1900.

[157] See Domansky and Reimann, Zeitschr. f. Heilk., 1901, and Herrick, Am. Jour. Med. Sci., vol. cxx, 1900.

Koslowoski[159] recommended Farrant's mounting fluid. This consists of equal parts of water, glycerin and saturated aqueous solution of arsenous acid (saturated by weeks of standing); to this gum arabic, ½ volume, is added and this allowed to stand (about three weeks) till all is dissolved. It is then filtered if necessary. The urine is mixed in a centrifuge tube with 1 cm. of 1% eosin or methyl violet, then centrifugalized and washed by centrifugalization till all the urine is removed. One drop of the sediment is then mounted on the slide with 1 drop of the above fixing fluid.

Bohland advises to wash the sediments with salt solution and then to add Müller's fluid. They are kept in this for 2 weeks, changing the fluid 2 or 3 times. The Müller's fluid is then decanted and the sediment washed in absolute alcohol until this is colorless.

Testicular Casts.—Casts have been described in cases of "spermatorrhea" which "can hardly be distinguished from renal casts except that the urine is otherwise normal. They are all in the first glass of the two-glass test and the presence in the same specimen of spermatozoa will indicate their origin. They are supposed to arise in the testicle." We have inquired of those with a very wide experience in the examination of prostatic secretions and they say they have never seen any such objects, although certain cylindrical cells may at first glance resemble true casts (see Fig. 64). Spermatozoa may often be found, active at first, together with all of the elements of unripe semen. They soon disintegrate. Such are found not only after coitus and pollution, but also after epileptic and other convulsive seizures.

Gonorrheal Threads, Clap Threads.—These threads occur in a late stage of acute gonorrhea and in chronic gleet after the exudate becomes very mucous and scanty and so collects in the longitudinal furrows of the mucosa. They may be from a few millimeters to one centimeter long and are yellow or white in color. Some, and these are found in very chronic cases, are narrow, delicate, transparent threads which consist of mucus in which are imbedded a few epithelial and still fewer pus-cells. Others are shorter, firmer and contain more cells, especially pus-cells. They settle at once to the bottom of the glass, but float up as fine, easily recognizable threads if the urine be agitated. They may coalesce in the course of time and so become unrecognizable.

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[158] Zeitschr. f. klin. Med., 1899, Bd. 38.

[159] Virchow's Arch., 1902, vol. clxxix, p. 161.

Tissue Fragments.—PORTIONS OF CARCINOMA have been found in the urine, especially from papillomatous cancers of the bladder, some of which were large enough to be sectioned for microscopic study. In some of these spindle-cells were found which enclosed hematoidin crystals and red blood-corpuscles. Fragments from renal cancers have been very rarely found. No mass of recognizable sarcoma tissue has as yet been recovered from the urine, but Rothschild[160] found a structureless mass in the urine of a case of giant-cell sarcoma of the kidney which was 5.2 cm. long, 0.5 cm. wide, firm, glossy and transparent. Masses of caseous matter are sometimes found in the urine in cases of renal tuberculosis. To demonstrate elastic tissue fibers the urine should be centrifugalized, acid added to dissolve the phosphates, the supernatant fluid decanted and the sediment then warmed with an equal amount of 10% KOH, which will destroy all but the elastic tissue. The specimen is then again centrifugalized and the sediment examined microscopically.

Other gross masses which are met with in the urine are mucous casts (see page 221) and the fibrin masses sometimes found in chyluria, hematuria and in inflammatory conditions, especially tuberculosis.

Pus-cells.—A few pus-cells might perhaps be expected in any normal urine, but the presence of many would mean inflammation of the urinary passages, of the kidney, or the rupture of an abscess into the urinary tract. The number of pus-cells varies enormously. As a rule, if from the cortex of the kidney they are few in number; if from the passages, many.

Hottinger found in a case of cystitis 150,000 leucocytes per cubic centimeter of urine, which would be a daily loss of about one per cent. of the total number of leucocytes present at any one time in the normal circulating blood.

The origin of the pus-cells in a specimen of urine may be indicated by other constituents present, *i.e.*, by the character of the epithelial cells also found, by the casts, etc. The sudden appearance of a large amount of pus usually means a ruptured abscess. The pus-cells in gonorrhea are often enclosed in threads of mucus, the so-called Tripperfaden (see page 273).

The urine of women usually contains pus from the vagina.

The pus in alkaline urine swells to a slimy, gelatinous mass, which is more slimy still since it usually is mixed with so much mucus. Microscopically, the pus-cells in very acid urine are so cloudy and shrunken that they are unrecognizable unless acetic acid is added to make their nuclei visible. In an alkaline urine they swell and become glassy but even then it is not easy to see their nuclei. In a weakly alkaline, amphoteric, or faintly acid urine they may remain well preserved for a long time and even show active ameboid motion. The diameter of leucocytes varies from 7 to 12μ and their nucleus is small, usually polymorphous. The nuclei of some are vesicular. It is hard to distinguish these last cells from renal epithelial cells unless the sediment be stained. Senator considered that many of the pus-cells in Bright's disease were mononuclear leucocytes.

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[160] Deutsches med. Wochenschr., 1901, No. 50.

In the urine sediments of 2 patients all the leucocytes were so drawn out that they resembled spindle epithelial cells. This probably was due to too long centrifugalization. It often, but that was not true of these cases, is due to the technic of making the slide specimen.

THE ALBUMIN OF A PURULENT SECRETION.—There will always be some albumin in solution in a urine with a pus sediment (and this cannot be removed by filtration). It often is important to decide whether more albumin is present than the pus serum can explain; that is, whether a true renal albuminuria also is present. If casts and renal epithelium are found, a cortical origin for some at least of the albumin may be assumed.

Posner's Method.—The total albumin of the filtered urine is first carefully estimated. The urine (a 24-hour specimen) is then well shaken and the leucocytes counted, using the leucocyte pipet and Toisson's solution and the ordinary blood-counting chamber. For each 100,000 leucocytes per 2 c.c. of urine, one may expect 0.1% albumin (Goldberg, 2 p.m.). If over 3000 cells per cubic millimeter are present the urine should be diluted with a 1 to 3% NaCl solution to bring the count near this figure. Kretschmer[161] believes this method valuable in following the treatment of a case.

Posner described also an easier method which has some value. The well-shaken urine is poured into a flat-bottomed beaker which rests on a sheet of ordinary printed paper until the urine so obscures the type that it can no longer be read. One can read through a layer of normal urine 8 cm. deep. If the type ceases to be legible when the urine layer is from 0.5 to 1 cm. deep we may conclude that 40,000 leucocytes per cubic centimeter are present; if 6 cm., 1000 leucocytes per cubic centimeter. This method is of some value in following the success of treatment.

Donné's Pus Test.—The supernatant urine of a specimen with a sediment which suggests pus is poured off and to the sediment is added a small piece or a strong solution of KOH or NaOH. If the sediment is pus it will be transformed to a viscid gelatinous mass which sticks to the glass.

Pus-cells will take a mahogany-brown color if treated with Lugol's solution.

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[161] Jour. A. M. A., 1917, vol. lxix, p. 1505.

Red Blood-corpuscles in the Urine in Cases Without True Hematuria.—Red blood-cells are present in the urine in cases of acute trauma, of stone anywhere along the urinary tract, in chronic passive congestion, in the hemorrhagic diathesis, after severe exercise, as long foot-races (Barach) and in many trivial conditions in which they would not be expected. Some of the cells are intact, others are shadows.

In concentrated urines the red blood-cells are crenated; in dilute, they are swollen or laked; in acid urines, intact; in alkaline, they are destroyed and form masses of yellowish-brown granules.

It is important to decide whether in any given case the red cells in the urine come from the cortex of the kidney or from some point along the urinary tract. That they come through the cortex may be assumed if many red blood-cells are sticking to casts or if true blood-casts also are present. An amount of blood sufficient for large clot formation seldom comes through the cortex and yet in rare cases of nephritis and in some cases of so-called renal epistaxis the urine may contain large blood-clots having the shape of the renal pelvis or of the ureter.

Gumrecht claimed that if many of the red cells were fragmented, that is, present as clumps of granules, one may assume that they were from the cortex since urea is the only constituent of the urine which could fragment them and the urea solution is strong enough (8%) to do this only in the cortex. Goldberg, however, believes that red cells can become fragmented in an infected bladder.

CONCRETIONS

Renal and Bladder Stones.—By renal stones are meant concretions from the pelvis of the kidney and the ureter. These vary in size from a grain of sand to large arborescent concretions which fill the whole renal pelvis. One weighed 1088 grains. The branches of these large stones may be hollow, thus furnishing a passage for the urine. The bladder concretions are single or multiple and vary greatly in size.

Uric Acid Concretions.—Of the renal stones those which are composed chiefly of uric acid are the most common. The size of those found in the bladder varies from that of a pea to that of a goose egg. They are always colored, their tint varying from a grayish-yellow to a yellowish or pale reddish-brown. Their surface is sometimes smooth and polished, sometimes rough and nodular. They are very hard, fracture easily and on cross-section show a crystalline structure and concentric arrangement of layers of different colors, which layers may be composed alternately of uric acid and some other salt, as CaOx. These stones burn without residue if pure; they give the murexid test; on the addition of NaOH they liberate but little ammonia· they are soluble in alkali, and from this solution, if acetic acid be added, crystals of uric acid will crystallize out. These crystals should be subjected to the murexid test.

TABLE IV

WHEN THE CONCRETION IS HEATED ON THE PLATINUM-FOIL THE POWDER

(HOFMEISTER'S TABLE)

Does not burn				Burns					
The powder + HCl				With flame			Without flame		
Does not effervesce			Effervesces.	The flame is yellow, continuous, odor of burning feathers. Insoluble in alcohol and ether; soluble in hot KOH, and reprecipitated white by acetic acid, with H_2S formation.	A yellow, clear continuous flame, odor of resin or shellac; the powder soluble in alcohol and ether.	Flame pale blue, burns for a short time with a characteristic sharp odor. The powder is soluble in NH_4OH and on evaporating hexagons are precipitated.	Does not give the murexid test. The powder is soluble in HNO_3 without gas effervescence, and this dried residue becomes orange when KOH added, then red on warming.	The powder gives the murexid test.	
The powder moderately burned + HCl									
Does not effervesce The native powder moistened with KOH		Effervesces.						The native powder on the addition of a little KOH in the cold	
Gives off much NH_3. The powder is soluble in acetic and HCl, and a crystalline precipitate formed with NH_4OH.	Gives off little or no NH_3. The powder is soluble in HCl and acetic acid—an amorphous precipitate falls with NH_4OH.							Gives off much NH_3.	Gives off little or no NH_3.
Triple phosphate	Neutral Ca or Mg phosphate	CaOx	$CaCO_3$	Fibrin	Fat	Cystin	Xanthin	Ammonium urate	Uric acid

Pure ammonium urate stones as primary concretions are found chiefly in the new-born, rarely in adults, although as secondary deposits they are very common. These stones are almost as soft as wax and when dry are clayey and easily powdered. They give the murexid test and if heated with NaOH much ammonia is liberated.

Calcium oxalate stones are, next to uric acid stones, the most common. Since they are the hardest and heaviest of stones they easily cause hemorrhages and so are often stained dark brown with blood pigment. They are soluble in HCl without gas formation, not in acetic acid, but if moderately heated the powder produced is soluble in acetic acid with gas evolution. If heated to a high temperature the powder reacts alkaline because of the $Ca(OH)_2$ formed. Kahn[162] who studied 16 renal stones decided that the most were composed of calcium oxalate; that they all contain some uric acid and urates; and that the shape, color and consistency of a stone give but little evidence of value as to its composition.

Phosphate Stones.—Pure phosphate stones are found rarely in the pelvis of the kidney. Those which are are small and consist of a mixture of the normal phosphates of the alkaline earths and the triple phosphate. In the bladder these stones may grow to very large size. The phosphates are also common ingredients of mixed stones. Phosphate stones often form around a foreign body. Their color varied—sometimes they are white or pale yellow, or purplish. They are soft, of light weight and have always a rough surface. The rare concretions of pure triple phosphates are small with a granular surface, upon which are often encrusted red crystals. Stones of acid calcium phosphate, which are rare, are white and of a beautiful crystalline structure. Phosphate stones if powdered do not burn, are soluble in acetic acid without gas formation and in this solution can be found phosphoric acid and the alkaline earths. They usually contain a great many organisms. The triple phosphate stones liberate much ammonia on the addition of NaOH.

Calcium carbonate stones which are rare in man, are chalky white in appearance and soluble in acid with gas formation.

Cystin stones are rare, but 106 cases having been reported.[163] Renal cystin concretions are seldom larger than a small pea, but those in the bladder may become as large as a hen's egg. They are light in weight, smooth and often so soft and wax-like in consistency that they may be crushed between the fingers. They have a smooth or ragged surface, are white or pale yellow in color, crystalline on cross-section, burn readily and perfectly on a platinum-foil with a bluish flame and are soluble in ammonia and recrystallized by acetic acid (for the other reactions of cystin, see page 256).

The very rare xanthin stones as a primary formation form occur especially in children. They vary in size from a pea to that of a hen's egg. They are pale white or yellowish-brown in color, rather hard, amorphous on cross section and on rubbing appear like wax. They burn without residue on the platinum-foil and the material of which they are composed gives the reactions of xanthin.

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[162] Arch. of Int. Med., January 15, 1913, vol. xi, p. 92.

[163] Kretschmer, Urolog. and Cutan. Rev., 1916, vol. xx, No. 1.

Fatty Concretions.—Only a few fatty concretions have been reported. These contained free fatty acid, neutral fat and much cholesterol. Some proved to be composed of the fat used in passing bougies.

Indigo.—Three stones, composed in part of indigo, are on record and yet indigo may be the nucleus of various other stones. They have a blue or bluish-gray surface.

Albumin.—One calculus said to consist of albumin is on record.

THE BACTERIOLOGY OF THE URINE

Unless extraordinary precautions are observed in collecting and preserving urine it soon contains hosts of bacteria of many varieties. Some of these it may have contained when it was voided, but the most are contaminations from the external genitalia, from the vessels which hold the urine and from the air. The urine is an excellent culture medium for many organisms and they soon render it unsuitable for chemical or microscopical study. Specimens to be studied chemically should be collected in clean bottles, chloroform, camphor, thymol, formaldehyde, etc., should be added at the very first and the specimen kept in an ice chest as much of the time as possible. Specimens to be studied microscopically should, whenever possible, be examined at once after it is voided. Especially if the specimen is to be studied bacteriologically, special technic is necessary in collecting and keeping it.

The Technic of Obtaining Specimens for Bacteriological Study.—To obtain a specimen for bacteriological study it is not always or often necessary to catheterize the male patient, especially if he is intelligent enough to observe the necessary precautions. The glans penis, and especially the edges of the urethral orifice, should be washed thoroughly with green soap and water and then with bichloride of mercury (1 : 1000). The anterior urethra is then thoroughly irrigated with bichloride of mercury (1 : 60,000). The patient then voids; the most of the urine is allowed to escape, thus completing the irrigation of the tract, and the last few cubic centimeters are collected in a sterile test-tube. A way preferred by some is to ask the patient to void into three sterile glasses. The third contains the specimen to be examined.

It is always necessary to catheterize the female patients. The external genitalia, and especially the orifice of the urethra, are well washed with green soap and water. The orifice of the urethra is then repeatedly mopped with sterile cotton pledgets soaked in sterile water, boracic acid, or mercuric chloride. At least 10 or 12 of these pledgets should be used. A sterile glass catheter is then inserted with care that it touches only the orifice of the urethra. The hands of the person introducing it should be surgically clean. Over the free end of the catheter should be fitted a rubber tube which will protect the tip of the catheter from contamination. This should be large enough to fit loosely and be about 4 inches long. After the most of the urine has escaped, this rubber tube is slipped off and the last small portion of urine collected in a sterile test-tube.

Bacterioscopic Examination of the Urine.—The examination of a smear made immediately after the specimen is obtained is by far the most important part of a bacteriological examination of the urine since in this way we may get a hint as to what culture media will best serve our purpose and may discover bacteria which will not grow on the media used as well as those which have already died. The smear may show a rich flora and the cultures the reverse.

Two of the reasons why smears of urinary sediments are not oftener studied for bacteria are that it is difficult to obtain good film preparations unless all the urea, which is very hygroscopic, has been previously washed out from the sediment; and secondly, that it is difficult to sediment bacteria by centrifugalization since the specific gravity of their bodies is nearly the same as that of urine. Nevertheless in the great majority of cases, especially if there is even a little pus present, one does get good smear preparations. One centrifugalizes the urine on a rapid machine until there is even a little sediment at the point of the tube, then quickly inverts the tube and allows all the urine to escape and drain. While still holding the tube in a perfectly vertical position a little of the sediment is scraped from the tip of the tube with a platinum loop. One must be careful to invert the tube to the vertical position quickly, and while the urine is draining and while obtaining the sediment not to incline the tube at all, else urine clinging to the sides may flow to the point and so add urinary salts to the smear.

But in case the urine is very clear and one wishes to obtain on a film preparation (but not for cultures) any organisms which may be present, one dilutes it with one, or even two, volumes of alcohol. This will so lower its specific gravity that practically all the organisms will be thrown by the centrifuge to the point of the tube. The urine thus diluted is very thoroughly centrifugalized, the supernatant fluid poured off, more alcohol or distilled water added, the contents of the tube well shaken and then again centrifugalized. (There is danger that many of the organisms will be left sticking to the sides of the tube rather than be thrown to its point. To obviate this in part at least, some allow a considerable amount of the mixture of urine and alcohol to sediment by gravity in a beaker and then centrifugalize the sediment.) The sediment will now be free from urea and satisfactory smear preparations on a glass slide or cover-glass can be made. It is often wise, in case but little sediment is present, to add a little egg albumin to stick the bacteria to the glass.

The smear preparation is first dried in the air, then passed slowly through the flame of a Bunsen burner or alcohol lamp three times and then stained.

BACTERIAL STAINS.—The bacterial stains in common use are solutions of the basic aniline dyes.

Löffler's Methylene Blue.—Saturated alcoholic solution of methylene e 30 c.c. and aqueous solution of KOH (1 : 10,000) 100 c.c.

The film is covered with this stain and heated over the flame for from 1 to 5 minutes. When no heat is used the staining will take much longer. The stain is then washed off with water, the film dried with blotting or filter paper and then mounted in Canada balsam. If on a slide the smear may be studied without the interposition of a cover-glass.

Saturated Aqueous Solution of Methylene Blue.—This is used as the above but stains a little more slowly.

Aniline Gentian Violet.—Aniline oil water is first made by adding exactly 2 c.c. of aniline oil to 98 c.c. of distilled water in a flask. This is shaken vigorously till as much as possible of the oil is dissolved and then filtered twice through the same paper. This fluid is kept in a dark-glass bottle and in a dark place.

To 75 c.c. of this aniline oil water are added 25 c.c. of a saturated alcoholic solution of gentian violet and the mixture filtered. This staining mixture is fairly permanent but should not be exposed to strong sunlight and should be occasionally filtered. Smears will stain readily in this in a few minutes. This is the stain used in Gram's method (see page 38).

Piffaud's Method[164] *of Staining Bacteria.*—This is a valuable method for determining the nature of a growth.

Cyanide blue solution:

Distilled water 100 parts;
Potassium cyanide (pure) 1 part;
Potassium carbonate (dry; pure) 0.5 part;
Rectified methylene blue 0.5 part.

A small drop of this stain is placed on the center of a slide, and then a loop of the growth well mixed with it. After 1 minute a clean cover-glass is dropped on this fluid and the excess of the moisture absorbed by pressing the cover-glass firmly with a piece of filter paper. In this way one dispenses with drying, heating and long staining.

Carbolfuchsin.—This contains:

Basic fuchsin 1 part;
Absolute alcohol 10 parts;
Carbolic acid solution (1 : 20) 100 parts.

This is a very powerful stain which when undiluted will stain bacteria in from ½ to 1 minute. Better results are obtained if it is diluted with from 5 to 10 volumes of water and left in contact with the smear for a few minutes. This is the stain used for the tubercle bacillus (see page 25).

BISMARCK BROWN (see page 38).

STAINING METHODS FOR ACID-FAST BACILLI (see page 25).

GRAM'S METHOD (see page 38).

CAPSULE STAINS (see page 32).

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[164] N. Y. Med. Jour., Nov. 2, 1907.

Spore Staining.—The film is first placed in a jar of chloroform for 2 minutes and then well washed in water. It is next placed in a 5% solution of chromic acid for from ½ to 2 minutes and again well washed with water. It is then covered with carbolfuchsin and heated in the same manner as if one were staining the tubercle bacillus (see page 25). The carbolfuchsin is not washed off with water, but with 1% sulphuric acid, or with methylated spirit (ethyl alcohol 9 parts, methyl alcohol 1 part) and left in this until decolorized. It is then washed in water, stained with a saturated aqueous solution of methylene blue for ½ minute, washed again in water, dried and mounted in balsam. The spores will retain the red stain, the bacilli will stain blue.

Flagellum Staining.—It is very difficult to get good specimens of stained flagella since only under certain conditions of growth, age, etc., can the flagella be demonstrated and even when the culture is a suitable one the flagella may very easily be injured by the technic used. The smear to be studied should always be made from a young agar culture incubated at 37° C. for from 12 to 18 hours. Kendall recommends to inoculate gently 5 c.c. of sterile water with enough of the above-mentioned growth to produce a faint turbidity in the upper half of the tube. This tube is then placed in a thermostat for 1 hour. This will allow the clumps to settle and the organisms to multiply a little. Without disturbing the fluid any more than one can help 2 or 3 loopfuls are placed on a clean cover-glass without attempting to spread the fluid at all and dried in a thermostat. The specimen is then fixed in a flame. (The cover-glass should be one which had been washed in a mixture of concentrated sulphuric acid 6 parts, potassium bichromate 6 parts and water 100 parts. It should then be washed thoroughly in water and kept until used in absolute alcohol.)

The staining methods are all of them so unsatisfactory that the best is usually the one with which the worker is most familiar.

Pitsfield's Method as Modified by Richard Muir.—The mordant consists of:

Tannic acid, 10% aqueous solution, filtered, 10 c.c;
Alum, saturated aqueous solution, 5 c.c;
Corrosive sublimate, saturated aqueous solution, 5 c.c;
Carbolfuchsin stain (see page 281) 5 c.c.

This is mixed thoroughly. A precipitate forms which is allowed to settle, or the fluid is centrifugalized, and the clear supernatant fluid removed with a pipet and kept in a clean bottle. This will keep for 1 or 2 weeks.

The stain consists of:

Alum, saturated aqueous solution, 10 c.c. and
Gentian violet, saturated alcoholic solution, 2 c.c.

This should not be more than 2 or 3 days old when used.

The film prepared as above described is covered with as much of the mordant as the cover-glass will hold and heated for about one minute over a flame just hot enough so that the fluid will steam gently. It is then well washed for about two minutes in running water and carefully dried over a flame. The specimen is then covered with the stain, heated, allowed to steam for about a minute, washed well in water, dried and mounted.

The smears of the sediment will give some clew as to the presence of organisms and as to the nature of some. In the case of certain organisms it is the only way we have to study them. This is especially true of the tubercle bacillus and the streptococci.

Bacillus tuberculosis (for staining methods, see page 25) may be found in the urine in cases of tuberculosis of the kidney or of any portion of the genito-urinary tract, providing that the kidney is still secreting urine and that the tuberculous focus has ulcerated into this tract. They appear, however, rather late in the disease and therefore are of little value in diagnosis. Bacillus tuberculosis is not infrequently found in the urine of tuberculous patients with apparently normal kidneys. They have been found in the urine of patients with miliary tuberculosis,[165] pulmonary tuberculosis and in fact with tuberculosis of any organ of the body. Some teach that their presence in the urine indicates tuberculosis of the urinary tract only when pus also is present in the urine, or when there are other signs of local tuberculosis.

Cases of sterile pyuria are often due to tuberculosis. There is no staining method which absolutely differentiates tubercle bacilli and the smegma bacilli in urine examinations. Cultural methods and animal inoculations are of value if positive. The urines to be examined should be obtained by catheterization using most careful aseptic precautions.

It is possible that some of the nonpathogenic acid-fast bacilli ingested with the food may appear in the urine but this must be rare.

To avoid smegma bacilli Young and Churchman (see page 284) advise thorough cleansing of the penis, rinsing it with large quantities of water, and careful irrigation of the anterior urethra. The urine, they say, should be passed into 3 glasses and only the third used in examination for tubercle bacilli. This technic, they believe, will fully exclude all smegma bacilli from the urine and any acid- and alcohol-fast bacilli present can be considered tubercle bacilli. In women the ureters must be catheterized.

Petroff's Method of Examination of the Urine.—The urine to be examined is acidified with 30% acetic acid and then 2% of its volume of a 5% solution of tannic acid added. The specimen is then put in the ice-chest for 24 hours. The precipitate can then be centrifugalized, redissolved with dilute acetic acid, centrifugalized and the sediment placed on slides and stained; or, the first precipitate may be treated with normal sodium hydroxide solution and cultivated.

The smegma bacilli are a group of organisms which grow in abundance on the external genitalia and wherever the secretions of the skin are allowed to accumulate. Their morphology and staining characteristics vary considerably. Some of the strains resemble so closely the tubercle bacilli both in morphology and in acid- and alcohol-fast staining reactions that they cannot be differentiated by this method. Twenty-one different stains have been published to differentiate these organisms from Bacillus tuberculosis, but in vain. It is fortunately not necessary as a rule to try to differentiate between them, since it is much easier to avoid them entirely by using care in obtaining the specimens, in which case any acid-alcohol-fast bacilli found can safely be called Bacillus tuberculosis.

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[165] Churchman, Am. Jour. Med. Sci., July, 1905.

Recent work[166] would tend to prove that smegma bacilli are difficult to cultivate directly from the patient. In fact, some hold that real smegma bacilli cannot be cultivated; others, that smegma contains two varieties of acid-fast organisms only one of which can be grown. Brereton and Smith found in the cases of 126 insane or uncleanly patients red staining bacilli in 85 (67.5%) if the specimens were decolorized by 25% sulphuric acid and in only 19 (22%) if methylene blue was used as a counterstain after decolorization. They were present in 13% if the specimens were decolorized by acid alcohol. In a second series of 20 men of ordinary cleanly habits, smegma bacilli were present in 13 (65%), if the specimens were decolorized with sulphuric acid only (25%), and in only 2 (10%) if the smears were counterstained.

Smears from the anterior urethra (fossa navicularis) of 24 patients showed (Young and Churchman) smegma bacilli in 11 (46%), while of 6 patients, they were found in the urine of 5. The urine in the bladder at necropsy and smears from the bladder wall were negative in 50 cases. The posterior urethra was negative for smegma bacilli in the 6 cases examined.

Streptococci also are much more safely searched for in smears from the sediment than by cultural methods.

In conclusion, no matter what the organism is which is present, one should always control his cultures by a preliminary bacterioscopic examination.

THE BACTERIOLOGY OF THE URINE, CULTURAL METHOD

The last portion of the urine voided is well centrifugalized, or first diluted with sterile water to dilute the urine and to wash the sediments (certainly no alcohol may be used), and cultures are made from the sediment.

The culture medium used will depend in great measure on the organisms suspected. When, however, the nature of the organism is not known, blood agar is the best medium to use, since all aërobic organisms which can be cultivated at all will grow on this. A few loopfuls of the urinary sediment are rubbed over the surface of this medium and the tubes then inoculated at 37° C. The different colonies can then be distinguished and transplantations made to suitable media.

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[166] Quoted from Brown, Jour. of A. M. A., 1915, lxiv, p. 886.

The MEDIA in common use are the following:

Nutrient Agar.—About 1500 c.c. of distilled water are heated over a furnace in a large metal pan while 15 gms. of agar are shredded and slowly added, together ith 2.5 gms. of Liebig's meat extract. The heating is continued, stirring at intervals until all the agar is dissolved. All floating scum is then skimmed off with a spoon. The pan is now removed from the fire, cooled slightly, and 10 gms. of peptone (Witte's) and 5 gms. of sodium chloride added little by little, stirring vigorously all the while to facilitate solution. The pan is then replaced on the fire and the contents boiled and stirred until all the peptone is dissolved. The fluid is then made just alkaline (to litmus) by the addition of a 5% solution of sodium hydrate. The pan is then removed from the fire and cooled to 60° F. To it is then added the whites of 2 eggs which in the meanwhile have been mixed with 150 c.c. of water. The pan is then replaced on the furnace and slowly heated until coagulation is complete. The fluid is not stirred while this coagulation is in progress. When coagulation is complete the pan and contents are weighed and enough water added to bring the weight of the contents up to just 1000 gms. (Twenty grams are allowed for the weight of each white of egg which will be filtered off.)

Meanwhile a large funnel with rubber tube and pinch-cock on the nozzle has been set up on a retort stand. In this is placed a well-moistened creased filter paper in a wire holder. The contents of the pan are now poured into the funnel through a strainer which will remove the coagulum. The medium is filtered at once into tubes or into a flask and sterilized for 7 minutes in an autoclave.

If the medium filters too slowly it is poured back into the pan, reheated, and then filtered through a fresh filter paper.

This is the medium on which the most of the common organisms are grown. It also is the basis of other special media.

Glycerin Agar.—This medium is similar to the above except that it contains from 6 to 8% of glycerin which is added after it is filtered. This fluid is tubed and sterilized in the autoclave for 7 minutes. It is superior to plain agar since many organisms which grow delicately on that grow well on this. This is true of streptococci, the meningococcus, the pneumococcus and the tubercle bacillus.

Blood Agar (Rosenau's Method).—This is one of the most valuable of media. About 100 sterile slant tubes of plain agar are first prepared. To a flask containing 50 c.c. of plain agar warmed to from 40° to 60° C., are added (under aseptic precautions) 15 c.c. of human blood. This is well mixed by shaking and from 1 to 2 c.c. are poured into each of the slant agar tubes which are then placed in such a position that this agar-blood mixture may harden as a uniform layer over the plain agar slant. In this way a little human blood will make a great many tubes. This technic must be aseptic to prevent contamination, since these tubes should not be sterilized again.

The tubes are then left in the thermostat for a few days to be sure that they are sterile.

On this medium will grow practically all aërobic organisms which can be cultivated at all. It is especially good for the gonococcus and Bacillus influenzæ.

Nutrient Gelatin.—Nutrient gelatin is made in practically the same way as is nutrient agar except that instead of agar one uses from 100 to 150 gms. of " gold leaf " gelatin. This medium should, however, be boiled as little as possible and should be sterilized in the autoclave for not over 5 minutes. (Of course tubes of this medium should not be placed in a thermostat kept at body temperature, but kept at room temperature or in a special thermostat the temperature of which does not rise above 22° C.

Litmus Milk.—About 100 c.c. of fresh milk are allowed to stand in the refrigerator for 5 hours and as much of the cream as possible removed. Then enough litmus tincture is added to give the milk a deep sky-blue color. The medium is then tubed and sterilized in the autoclave for 7 minutes.

Bouillon.—The formula is:

Liebig's meat extract 2.5 gms;
Peptone (Witte's) 10 gms;
Sodium chloride 5 gms;
Water, distilled, 1000 c.c.

The steps for making this medium are practically the same as those for plain agar. To the boiling water is added the meat extract and the boiling then continued for 5 minutes. The pan is then cooled down a little, the peptone and salt added slowly and then dissolved by boiling and the fluid made alkaline as described above. This medium when finished is first poured into a flask and sterilized in an autoclave for 5 minutes, then cooled, filtered twice through the same paper, then tubed and sterilized again in the autoclave for 5 minutes.

Löffler's Blood-serum Mixture (see page 37).

Among the more important organisms which may be encountered in the urine are the following:

Bacillus Coli Communis.—The colon group includes 20 or 30 very similar varieties which usually are grouped under this one name. It is a short organism, the majority from 1 to 2μ long and 0.5μ thick. Some, however, are so short as to resemble cocci and others are over 5μ long. They often are seen in pairs. The most of the varieties are sluggishly motile, although this may not be evident in cultures over 24 hours old, while other strains are very motile. Its flagella are numerous and laterally placed. It is easily stained by all the usual aniline dyes and is decolorized by the Gram method. It does not produce spores. The organisms with unstained portions resembling spores are involution forms. It grows rapidly on all ordinary media at room as well as at body temperature and in a characteristic way. The growth on the surface of agar is abundant, thick, moist and spreads rapidly. The deep colonies are very circumscribed and opaque, with a well developed nucleus. This bacillus does not liquefy gelatin and does not spread on the surface of this medium as it does on agar. The surface colonies on gelatin have a nucleus and well-defined granular or striated, refractive halo. It turns litmus milk rapidly acid (within 18 hours), coagulates it (in from 4 to 30 days) and does not later digest the clot. The growth on potato is abundant and visible. It ferments almost every known carbohydrate, but especially glucose, lactose and saccharose and with abundant gas production. It produces indol. The colon bacillus is almost ubiquitous. It is the prevailing organism of the lower part of the small intestine and the colon and is almost universal in soil, water, food, etc. It is a mildly pathogenic and pyogenic organism. It is the organism found most frequently in infections of the urinary tract.

Bacillus Typhosus.—The typhoid bacillus is a long slender organism measuring usually from 2 to 4μ in length and about 0.5μ in thickness. It is actively motilar. Its flagella are more numerous (from 5 to 50) and somewhat longer than those of Bacillus coli communis. It is easily stained by all the aniline dyes and decolorizes by Gram's method. It does not produce spores. It grows on all ordinary media. The growth on agar is thin and translucent with slightly spreading dentate or leaf-like edges. The deep colonies have sharply defined edges and a distinct nucleus. Bacillus typhosus does not liquefy gelatin. Its growth on potato is often, but by no means always, invisible. (The colon bacillus grows on potato as a distinct brownish scum and the typhoid bacillus may also. This seems to depend on the potato used.) A most important feature is the reaction of this bacillus to litmus milk. A very slight acidity is first produced, distinct, but never enough to coagulate the milk not even in weeks. This slight acidity may be permanent although some strains later furnish enough alkali to change the reaction back to neutral, or even to alkaline. Certain carbohydrates, including dextrose, levulose, maltose and mannites are fermented to the point of acidity, but none with gas production. Saccharose and lactose are not at all affected. It does not form indol.

For the certain recognition of this bacillus its agglutination in the very dilute serum (1 : 50 to 1 : 1000) of a patient or animal immune to Bacillus typhosus is necessary (see page 565).

This organism is present in the urine of ⅓ of all cases (see page 291) of typhoid fever during the fever and in some cases for years afterward.

The Paratyphosus Group of Organisms.—The more carefully the cases of " typhoid fever " are studied bacteriologically the more numerous those are found to be which are due not to Bacillus typhosus but to very similar bacilli which resemble also, in some essential respects, Bacillus coli communis. Those which stand nearest this latter organism are called " paracolon " bacilli, those nearer Eberth's bacillus, " paratyphoid." There is no one Bacillus paratyphosus, but a group. In the clinical laboratories at least eleven such organisms have been isolated, although for all practical purposes of diagnosis we recognize but 2: Bacillus paratyphosus *A*, and Bacillus paratyphosus *B*. The court of last appeal in the recognition of these bacilli is the serum agglutination test, using the serum of an animal immunized to the organism in question. This is fairly satisfactory although they show a rather marked group reaction. Clinically we note whether or not the patient's blood will agglutinate one of them.

The characteristic culture features of the paratyphoid bacilli are their reaction to milk and to sugars. All resemble Bacillus typhosus in that they produce first a slight acidity in milk, but they all (and this may take weeks) finally change the reaction back to alkaline. The attempt to divide the group into the " *A* " group of organisms which keep the milk acid and the " *B* " group which change it back to alkaline seems unjustified.[167] Again, these all ferment glucose with gas production. Some ferment lactose, also some saccharose, but none all of these three sugars.

BACILLUS LACTIS AËROGENES.—Bacillus lactis aërogenes is a short, thick (about 2μ thick) non-motile, encapsulated bacillus, seen usually in pairs and sometimes in chains. It often shows rather marked polar staining. It is decolorized by Gram's method. It is not a spore-producing organism. It produces on all media a luxuriant, viscid, slimy growth which resembles in many ways that of Bacillus coli communis. It does not liquefy gelatin. It ferments practically all the carbohydrates rapidly and with abundant gas production. This organism has been the subject of much dispute. Some do not distinguish it from Bacillus coli communis, some group it with the capsulated group. Some strains of this organism cannot be distinguished from Bacillus capsulatus of Friedländer (Bacillus pneumoniæ).

This bacillus is a normal inhabitant of the upper part of the small intestine where it is the predominating organism. Some believe it the cause of certain cases of cystitis.

BACILLUS ALKALIGENES.—This bacillus is a normal inhabitant of the intestinal tract and has been often mistaken for Bacillus typhosus. It is a long, slender bacillus from 2 to 3μ long and 0.5μ thick, which grows singly, in pairs, or in chains and which is actively motile.

It grows on all culture media. It does not liquefy gelatin. Its most characteristic cultural reaction is the production of an intense alkaline reaction in litmus milk without previous acid production. It ferments carbohydrates without producing an acid reaction and without gas production and is the only one of the common intestinal flora which grows only in the open end of the fermentation tubes and not at all in the closed end.

THE PROTEUS GROUP.—The members of this group, among which are Proteus vulgaris, Proteus mirabilis, Proteus zenkeri, Proteus zoffii, and other strains with but minor differences, are the most important agents of putrefaction. While secondary invaders as a rule, they are in certain cases of cystitis the pathogenic organism. In fact this is almost the only organism which when introduced into a normal bladder will set up a cystitis. They are the most important organisms in the production of a " sapremia," or intoxication from the products of decomposition, as in cases of retained placenta. They are short, slender, actively (even violently) motile bacilli with terminal flagella.

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[167] Ford, Medical News, June 17, 1905.

This description of the organism applies only to those obtained from fresh cultures. If cultures a little older are examined one will find in the smears cocci, bacilli of all sizes, spirilla, etc., suggesting a badly contaminated culture. But if fresh transplantations be made from this culture and examined early the organisms will be found to be bacilli of uniform morphology. This marked polymorphism is due to the tendency of this organism to produce involution forms. It is decolorized (?) by Gram's method. It grows well at room temperature. The colonies on agar spread rapidly in a characteristic manner since the edges send out peculiar hair-like projections which make the colonies look like tufts of moss. Proteus vulgaris and mirabilis liquefy gelatin (the former rapidly, the latter slowly), the blood-serum rapidly; Proteus zenkeri and zoffii do not. Milk is coagulated and clot then digested, the reaction remaining all the while alkaline. The strains of the proteus groups differ somewhat in their ability to ferment sugars. The formerly much-talked-of Bacterium termo is possibly one of this or of a nearly related group.

BACILLUS PYOCYANEUS.—Bacillus pyocyaneus is a small organism about 2μ long and 0.5μ thick, actively motile, which is easily stained by all ordinary bacterial stains and which shows often a marked bipolar staining. It decolorizes by Gram's method. It grows rapidly on all ordinary media and will crowd out any other organism which happens to grow along with it. Its important cultural characteristics are that it will not split up carbohydrates, that it liquefies gelatin and blood-serum rapidly, that it coagulates litmus milk rapidly, decolorizing the litmus and then digesting the milk clot with the reaction alkaline and, lastly, that it produces 2 pigments in its growth—a non-specific fluorescent pigment and a specific bluish-green pigment called " pyocyanin."

This is a very common organism often met with in the intestine and on the skin, especially in the folds of the axillæ and groin. It is the pyogenic bacillus which produces blue pus; it sometimes is the organism of septicemia of children; but its most important rôle as a pathogenic organism is as the cause of cystitis and ascending genito-urinary infections.

BACILLUS AËROGENES CAPSULATUS.—This is one of the most widely distributed of pathogenic organisms. It is a constant inhabitant of the intestine of man and of animals and is commonly present in the soil, water, milk, etc.

This is a large bacillus, from 1.5 to 6μ long and 1μ thick. It is non-motile, is encapsulated and produces spores in the animal body and when grown on blood-serum. It is easily stained by all the ordinary stains and does not decolorize by Gram's method.

It is a pure anërobe, growing only in the complete absence of oxygen. It grows best (under anërobic conditions) at 37° C. in the depth of solid media as grayish-white or brownish colonies with fine feathery or hair-like projections from their edges. It ferments sugars easily. Litmus milk is coagulated, decolorized and the clot later digested.

Since it is met with very often in mixed infections the best way to cultivate it is to heat the material containing the mixture of organisms at 80° C. for a few minutes to kill off all but the spores. Plate cultures are then made and cultivated anërobically. The most of the organisms in such a mixture are not spore producers and so will be killed off leaving but a few forms alive of which this will be one.

A still better way to isolate this organism is to inoculate a rabbit intravenously with the material containing the mixture of organisms. After 5 minutes the animal is killed and placed in the thermostat for from 6 to 8 hours or left in a room temperature for 18 to 24 hours. The animal will during this time become much distended with gas. Bacillus aërogenes capsulatus can now be obtained from the blood in almost pure culture.

This organism is one of the most important of the pathogenic bacteria. Its infections are extremely grave. Those of the genito-urinary tract are not infrequently the result of dirty technic in obstetrics.

BACILLUS TETANI.—The tetanus bacillus is a slender organism about 4 or 5μ long and 0.5μ thick. It is a motile bacillus with a great many very long, slender flagella. It is a spore-producing bacillus and since the spores have a diameter 3 or 4 times that of the bacillus and are usually situated at the end of the bacillus the drum-stick shape of the sporulated organism is characteristic. Those with a spore at each end resemble a dumb-bell. These spores are very resistant to heat and are not killed by a temperature of 80° C. for 1 hour.

This bacillus is easily stained by all the ordinary bacterial stains, the body of the bacillus taking an unusually uniform stain. It is not decolorized by Gram's method.

The tetanus bacillus is a perfect anërobe and can be isolated only with extreme difficulty. Fortunately it is not necessary to grow it since its appearance in smears is quite characteristic.

It is one of the most ubiquitous and important of pathogenic organisms. Its normal habitat would seem to be the intestine of cattle and so may be found wherever the soil is contaminated with manure.

The bacteriological study of the urine is often important in the diagnosis of septicemia, acute nephritis, pyelitis, ureteritis, cystitis, prostatitis and urethritis. In case of SEPTICEMIA one would try first to isolate the organism from the blood. Since the importance of bacilluria in the spread of diseases has been recognized, the presence of organisms in the urine has aroused a new interest. It is now known that Bacillus typhosus can be found in the urine of about one-third of the cases of typhoid fever during the attack. Sometimes these bacilli are few in number, sometimes so numerous, even 500,000,000 in each cubic centimeter, that they actually cloud the fresh urine. This bacilluria may clear up with the fever but it also may persist for years without giving rise to any local symptoms which might warn these "chronic bacillus carriers" that they are spreading this disease far and wide.

In other forms of septicemia also the invading organism is often found in the urine. The presence in the urine of Bacillus tuberculosis in cases of chronic tuberculosis, especially of the acute miliary form, has already been mentioned (see page 283). In some cases of streptococcus infection the fresh urine on the day of death is turbid because of the streptococci it contains.

Infectious Nephritis.—So many cases of acute nephritis date to an acute infectious disease, such as pneumonia, tonsillitis, influenza, typhoid fever, etc. (to say nothing of scarlet fever, measles, etc.—diseases the specific organisms of which are not yet known) or to an acute infection, as in a recent case of streptococcus infection of the arm complicated by acute nephritis, otitis media leading to meningitis, etc., that it is not unreasonable to believe that the nephritis is due to the same organism as that which causes the primary infection and that cultures from the urine therefore may help discover this germ. Especially may this be true of the interesting cases of unilateral nephritis.

Acute pyelitis is due to an infection which usually reaches the pelvis of the kidney from the blood stream but which theoretically may be an ascending infection secondary to a cystitis, or possibly one which has traveled along the lymphatics. Fortunately now it is an easy matter to catheterize the ureters of patients and so get urine for cultures directly from the pelvis of each kidney. In a pyuria of renal origin if the cultures are sterile the cause is usually tuberculosis of the kidney. We will speak of the bacteriological findings in pyelitis in connection with those in cystitis.

It may be mentioned at this point that a pyelitis without localizing symptoms is quite common and that a pyelitis may continue for considerable time before a cystitis begins.

Cystitis.—Cystitis is an inflammation of the urinary bladder due to some pathogenic organism. This bladder when normal is very resistant to infection and organisms introduced into it soon disappear unless some predisposing factor has lowered the resistance of its wall. The 2 exceptions to this general rule are Bacillus tuberculosis and proteus, which if introduced into a normal bladder can set up a cystitis. Among the conditions favoring infection may be mentioned: calculus; posterior gonorrheal urethritis; frequent catheterizations; the retention of urine from any cause (very important) such as childbirth, enlarged prostate, urethral stricture, spinal cord disease and prolonged narcosis; and, finally, infection of the rectum with secondary involvement of the bladder.

Tuberculous Cystitis.—Among the forms of cystitis the cases due to tuberculosis form so well-defined a group that they deserve special mention. A primary tuberculous cystitis is a rare condition. This infection usually descends from the kidney or ascends from the genital tract. The descending cases are particularly important since all the symptoms often are vesical and the renal disease, even though of extreme grade, may be unsuspected until cystoscopic examination suggests a higher origin and ureteral catheterization determines the source and nature of the pus.

Tuberculous cystitis is a disease especially of young adults. It begins insidiously and may last for years. There is usually in well-developed cases increased frequency of micturition, but not always. The urine is acid and contains pus, often considerable, a condition which may have escaped the patient's attention. Cultures made from it are sterile. There are 2 general rules of value. All persistant, acid pyurias in the young are presumably tuberculous until the contrary is proven (Kelly); and all sterile (in ordinary cultures) pyurias are either tuberculous or gonorrheal in nature (see page 294). It is easier to demonstrate the tubercle bacillus (see page 283) in tuberculous cases than the gonococcus in those cases.

There is an interesting group of very early cases of tuberculous cystitis with practically no pus at all in the urine, with slightly increased frequency of micturition and slight, transitory hematuria, which may persist for a few days and then not reappear for weeks. The urine is clear and rather highly colored, but the last few cubic centimeters of the voiding usually contain a few drops of blood.

In advanced cases of tuberculous cystitis the urine remains acid, the pyuria is marked, tubercle bacilli are easy to demonstrate and there is persistent or recurring hematuria. Following a secondary infection, and sooner or later this is quite certain to develop, the pus usually increases in amount and the urine often becomes alkaline.

It is important to remember that cases of tuberculous prostatitis and ureteritis may so resemble tuberculous cystitis that only a cystoscopic examination will localize the lesion.

Cystitis due to Organisms other than Bacillus Tuberculosis.—Among the organisms found in cystitis are Bacillus coli communis, Staphylococcus aureus, Staphlycoccus albus, Streptococcus pyogenes, Bacillus proteus vulgaris, Bacillus pyocyaneus, Bacillus typhosus, Bacillus lactis aërogenes and others. In many cases it is difficult to determine just how important in the etiology of the cystitis is the organism isolated in cultures. Many are certainly secondary invaders and harmless saprophytes.

Bacillus coli communis is the most important of the above list. It causes a very chronic cystitis.

It must be very difficult indeed for the gonococcus to gain a primary foothold in the bladder, for in practically every one of the very numerous cases of gonorrheal posterior urethritis this organism must frequently enter the bladder. The region of the trigone would seem the most susceptible spot in the bladder to this infection, which once developed may be quite stubborn. Other cases of definite gonorrheal cystititis develop as part of an acute urethritis and clear up rapidly. The gonorrheal and tuberculous infections are the only 2 which seem exceptions to the general rule that pyuria is an almost invariable symptom of cystitis. It is very difficult to demonstrate the gonococcus in these cases, and secondary pyogenic infections, a not uncommon sequel of gonorrheal cystitis, usually mask the picture.

Proteus cystitis is a common and very distressing form. Bacillus proteus seems to be almost the only organism which when introduced into a normal bladder will set up a cystitis (Melchoir). The urine in these cases is very alkaline and the abundant pus is transformed into a ropy, sticky, mucoid mass.

Streptococcus cystititis is often a very severe form, but sometimes is mild. Bacillus lactis aërogenes is believed to be a much commoner cause of cystitis than statistics would lead one to expect.

The catheterized urine of cases of cystitis practically always contains pus. Certain cases of tuberculosis and of gonorrhea are exceptions to this rule. As mentioned above the kidney should always be excluded as the source of much of this pus, especially if there is much fever or if there is more albumin than the pus alone would explain. As a rule the most of the pus appears in the first and the least in the second of the 3-glass test, but this holds true only if the patient had been resting before voiding. If the reaction of the urine is acid the pus-cells often are well preserved, sometimes ameboid, and settle as a granular layer on the bottom of the glass; when very alkaline, the pus is transformed into a sticky, ropy, mucoid mass.

The reaction of the urine in the tuberculous cases is usually acid until secondary infection by proteus or a streptococcus occurs. In chronic cystititis due to the colon bacillus, Staphylococcus albus and other organisms with slight virulence, the urine may be either acid or alkaline. In the proteus and streptococcus cases the urine is alkaline, the phosphates are precipitated, the pus-cells unrecognizable and the odor of the urine foul.

Red blood-cells are numerous in the urine of cases of acute cystitis, their number varying with the acuteness of the attack. They are uniformly distributed throughout the urine. The hemorrhages from the bladder wall are slight. The larger hemorrhages come from the kidney, from vesical tumors, or especially from the prostatic urethra. In cases of posterior urethritis the blood may continuously ooze back into the bladder and when these patients void the urine is at first bloody and at the end of the voiding pure blood.

For mention of epithelial cells in the urine in cystitis, see page 262.

In "membranous," "exfoliative," "croupous," "diphtheritic," or "desquamative" cystitis the patient passes flakes, masses, or moulds of a tough fibrinous membrane containing much degenerated epithelium. These masses are supposed to be the results of necrosis of the inner layers of the bladder wall. In gangrenous cystitis fragments of the epithelial and muscular coats of the bladder are expelled in the urine. In hemorrhagic cystitis there may be much bloody infiltration of the bladder wall. Clinically these severe forms of cystitis are very rare. They occur often enough after the traumatic or operative opening of the bladder and as terminal events.

Bacteriuria.—When a bacteriuria is present the urine when voided may contain so many organisms that it is actually cloudy. The symptoms of these cases often suggest a severe cystitis and yet cystoscopic examination may show the bladder normal, although later a mild cystitis is almost certain to develop.

A transitory bacteriuria often follows massage of the prostate gland. It begins within a few hours after this procedure and may last 1 or 2 days. There are no symptoms. These organisms are supposed to come originally from the rectum.

The cases of persistent bacteriuria fall into two groups. The first is of renal origin, already mentioned on page 291, and due usually to Bacillus typhosus, Bacillus coli, etc. The second group included those cases which are secondary to a posterior urethritis and prostatitis. While the foci of infection are in these organs the organisms multiply in the bladder and are so numerous that they may cloud the urine. Very little pus is present in the urine of these patients.

In gonococcus, typhoid, and colon bacteriuria the urine remains acid; in streptococcus and staphylococcus bacteriuria its reaction may be acid, neutral, or alkaline; in the Staphylococcus albus bacteriuria, however, the urine is usually alkaline.

INFECTIONS OF THE URETHRA AND EXTERNAL GENITAL ORGANS

In this connection it will be necessary to describe 3 very important organisms not mentioned in the preceding pages.

The Gonococcus.—The gonococcus is so important an organism that the medical student should be skilled in its recognition. Formerly supposed to be the cause of unimportant, transitory, local infections, it is now known to be one of the most destructive of organisms.

The gonococcus (see Fig. 60) is a coccus about 1μ in diameter, which occurs, as a rule, in pairs. The proximal edges of these diplococci are flattened, giving the organism the well-known biscuit shape. In smears of gonorrheal pus the organisms are found chiefly inside pus and epithelial cells, or in masses on their surface; but some are free. It stains well in all the ordinary bacterial stains, but especially in methylene blue, and discolorizes by Gram's method. (This organism in smears of pus, however, decolorizes so slowly that it may be necessary to leave the specimen in alcohol for 10 minutes.) The gonococcus grows only on media containing some human proteid, as human blood agar and blood-serum, but agar mixed with ascitic fluid or hydrocele fluid will be satisfactory, or a medium made up of urine, blood-serum and agar. Its growth on proper media is a thin, moist homogeneous layer which dies in a few days. Cultivated under the best of conditions and transplanted frequently, the gonococcus will survive but for a few generations. It is very susceptible to changes in temperature. It dies in a few hours in pus at room temperature and is quickly killed by a temperature of 40° or 41° C. It is killed rapidly by drying. For its recognition it is necessary to assure oneself that it occurs as biscuit-shaped diplococci, in clusters or clumps, some of them at least intracellular, that at least 1 cell found contains many such organisms, that it decolorizes by Gram's methods, and that it grows only on the above-mentioned media.

The gonococcus is the important causative agent of urethritis and periurethritis, prostatitis and infection of the connecting ducts and glands of the genito-urinary system, the seminal vesicle, prostate, epididymis, bladder, Bartholin's glands, vagina, uterus, tubes, etc.; it causes an eczematous skin eruption about the genitals; it is an important cause of proctitis, peritonitis, meningitis, endocarditis and especially of conjunctivitis, arthritis and septicemia. A definite discharge is not a necessary indication of possible gonorrheal infection, for it may produce very little pus, as in the vaginitis of infants and in chronic arthritis.

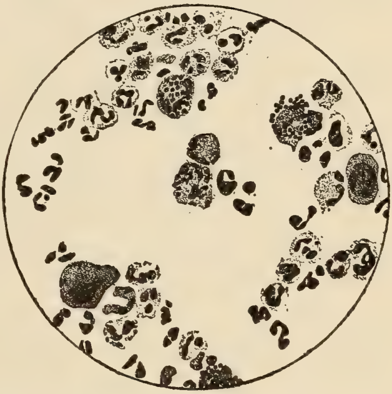

FIG. 60.—Spread of pus containing gonococci. (Wilson.)

Much has been written of organisms often found in the normal urethra which morphologically "are exactly similar to the gonococcus, and which also decolorize by Gram's method." These, however, will grow easily on ordinary culture media. One would not be likely to mistake these if he remembers that the gonococcus is found in groups or clumps of organisms in a smear, while one would find but 1 or 2 of the pseudogonococci on a slide.

Acute Anterior Urethritis.—The discharge in a case of acute urethritis during the first few hours is scanty, resembles dilute milk or starch solution and consists chiefly of serum, epithelial cells, a very few leucocytes, often a few red blood-cells and a few gonococci, the most of which are extracellular. After a few hours, however, the discharge becomes more abundant, yellow, creamy in consistency and is then an almost pure bloody pus in which gonococci are easily found. During the first very few days of the infection the gonococcus will be the only organism found, but later the ordinary rich urethral flora returns.

In an untreated case, which may clear up spontaneously in from 4 to 6 weeks, the discharge again becomes starchy, more and more scanty and consists then of abundant mucus with fewer pus-cells, but with more and more epithelial cells. The gonococci meanwhile become fewer and more difficult to find. Finally the discharge is almost pure mucus which contains no pus or gonococci. In case the disease had not extended beyond the anterior urethra the patient is now well, but only too often the infection extends to the posterior urethra and adjacent structures, in which case secondary infections by pyogenic organisms are common and these modify the exudates.

In *posterior urethritis* the discharge is often profuse, but since it is restrained by the compressor urethra muscle it all must flow back into the bladder and be voided with the urine. The discharge from the anterior urethra may at this time be very scanty. If this patient passes his urine in two portions the discharge then present in both posterior and anterior urethra will be washed out with the first portion of urine while the second glass will contain the pus which has been flowing back into the bladder; yet this second glass should not contain as much pus as does the first glass. If the amount of exudate from the posterior portion be small the second specimen of urine may be clear. If the anterior urethra is first well irrigated with boric acid solution and then the patient voids into 2 glasses, the presence of pus in the first will indicate a posterior urethritis. The best time to try this test is with the first voiding in the morning.

The sequelæ of posterior urethritis are prostatitis, vesiculitis, epididymitis and cystitis. In very acute posterior urethritis the frequent and excessively painful micturition is a very distressing symptom. The whole urine may be colored by the blood which is constantly flowing back into the bladder and at the end of micturition a few drops or more of pure blood often flow from the urethra (" terminal hematuria").

If a chronic urethritis follows an acute, the discharge may be continuous and fairly profuse or very scanty. In the latter case there may be only enough exudate to glue together the lips of the urethral orifice but as a rule there is a little exudate which consists of shreds of mucus enclosing a few pus, but more epithelial, cells. This discharge should be carefully distinguished from the glairy discharge which follows an acute urethritis. Smears should be carefully studied for pus-cells and gonococci. This exudate is washed out of the urethra as Tripperfaden (see page 273). It is very difficult to demonstrate the gonococcus in such an exudate.

These " clap threads " when long, translucent and branching are made up mainly of mucus which is washed out of folds in the urethral mucosa;

others are short, thick, tack-shaped and sink quickly to the bottom of the glass. These contain considerable pus. They are supposed to come from the urethral crypts. Some of the shreds from the posterior urethra are short, slender, delicate, and comma-shaped. These are from the prostatic excretory ducts (Fürbinger's hooks). These shreds should be carefully examined for gonococci.

In other cases of chronic urethritis the discharge is an abundant, thick pus in which pyogenic organisms also may be present, but not always. Still other cases have an intermittent discharge of muco-pus or almost pure mucus.

If the patient immediately after irrigating the anterior urethra voids into 3 glasses and shreds are found in any of the glasses there surely is present a chronic posterior urethritis. In these cases the whole volume of urine may be slightly cloudy because of the exudate which is constantly flowing back into the bladder.

In women an acute urethritis is of briefer duration and less apt to become chronic than in men. (Many deny this.) Next to the urethra, the cervix in women is the most common focus of gonorrheal infection. The cervical discharge is at first slimy and blood-stained and later a milky pus. In this location, especially, is the infection apt to become latent and chronic, and its only sign a viscid catarrhal mucous discharge.

The discharge in cases of vulvitis and vaginitis is, because of secondary infections, often a profuse and very fetid pus.

Non-specific Urethritis.—Not all cases of urethritis are due to the gonococcus. Some are due to various other organisms, the colon bacillus, the diphtheria bacillus, streptococci, staphylococci, etc.

Smears of the exudate of the cases not gonorrheal in nature will show the presence of these other organisms in great numbers. Nevertheless, it is well to remember the frequency with which secondary infections complicate a true gonorrhea and the difficulty one often has to find the gonococcus when it is present.

In these non-specific cases the exudate is purulent, but not profuse and the cases are mild and respond readily to treatment.

Bacteriorrhea.—Some patients have as urethral discharge a thin opaque fluid, which microscopically consists almost entirely of mucus and saprophytic bacteria of all varieties, but no pus-cells. These patients have no other symptoms. The discharge clears up quickly under treatment. This condition is called bacteriorrhea.

Prostatitis.—Prostatitis may be due to the extension of an infection from the urethra or from the rectum, or to an hematogenous infection.

The diagnosis of acute prostatitis is more a matter of physical than urinary examination. In chronic prostatitis [168] the diagnosis is made by physical examination but also by the examination of the prostatic fluid which one obtains by massaging the prostate gland after the urethra has been well irrigated.

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[168] For complete description see Young, Johns Hopkins Hosp. Reports, 1906, vol. xiii, p. 302.

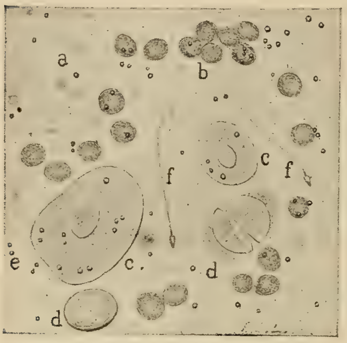

Fig. 61.—Prostatic fluid. (X 400.) *a*, lecithin globules; *b*, pus-cells; *c*, epithelial cells; *d*, corpora amylacea; *e*, free granules from epithelial cells; *f*, spermatozoa.

The normal prostatic fluid, which is a thin, bluish, skim-milk-like fluid, is described on page 308 (Fig. 61). The fluid in a case of chronic prostatitis may contain none, some, or all, of the normal constituents of this fluid and in varying amounts. No diagnostic or prognostic value can as yet be ascribed to the presence or relative amounts of these normal constituents. The abnormal element of greatest importance is pus. In some cases at times, and especially on the first examination, no pus-cells will be found and yet later, many. The amount of pus present bears a fairly direct relation to the extent of prostatic involvement. Red cells are sometimes abundant. Spermatozoa, active or immobile, are found in varying numbers. In chronic prostatitis the fluid is always alkaline to litmus.

When there is so much prostatic fluid that a discharge follows urination or defecation the condition is called "prostatorrhea" (also called "spermatorrhea"). This usually indicates a rather mild prostatitis.

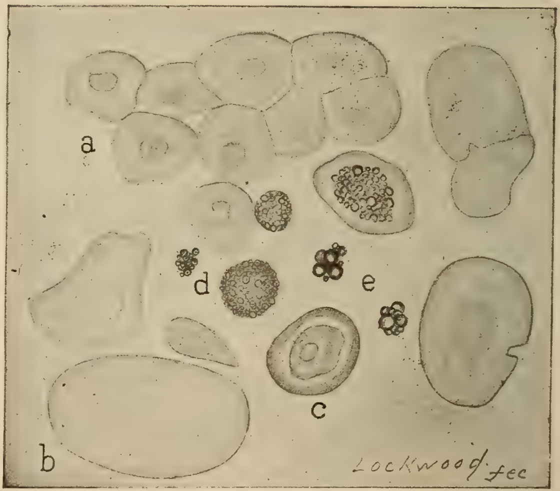

Fig. 62.—Prostatic fluid: *a*, epithelial cells; *b*, clear epithelial cells, from seminal vesicles (?); *c*, corpus amylaceum; *d*, "granular cells" with droplets resembling myelin; *e*, "granular cells" with fat droplets.

The fluid which can be expressed from the seminal vesicles is a thick and gelatinous secretion which resembles boiled tapioca or sago. This fluid sinks in the urine. Its chief constituents are "mucin globules," large unformed masses resembling large non-nucleated epithelial cells (Fig. 62, *b*), spermatozoa (Fig. 63 represents a large mucin globule full of spermatozoa), pigmented epithelial cells, and small finely and coarsely granular non-nucleated epithelial cells. Some cells might be mistaken for casts (see Fig. 64).

For purposes of instruction the students may well use the 7-glass test in the differential diagnosis of chronic inflammatory lesions of the urethra, prostate, seminal vesicles and bladder, although this test is not often used in practice. The patient compresses the urethra far back at the root of the penis (at the suspensory ligament) while the anterior urethra is irrigated by means of a long irrigating tube. The fluid is caught in 2 glasses. The first, I ^{1}, will contain the shreds, if any are present, the second, I ^{2}, should be perfectly clear. The patient's fingers are then removed and the tube carried back as far as the deeper part of the bulbous urethra. The washing is again caught in 2 glasses. The first, I ^{3}, will contain the shreds from the bulbous urethra (if any are there), the second, I ^{4}, should be clear. The urine is then voided into 3 glasses, I ^{5}, I ^{6}, I ^{7}, which will contain bladder urine, and mixed uniformly in each the exudate of posterior urethritis which has flowed back into the bladder between voidings. In addition to this:

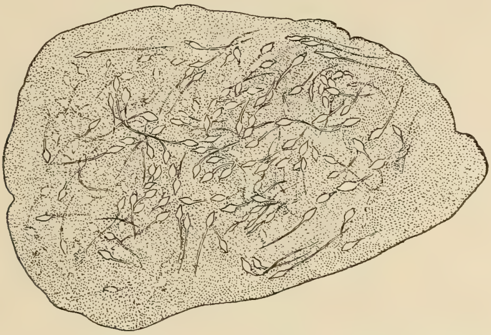

Fig. 63.—Mass of mucus filled with spermatozoa from urine catheterized at death. × 400.

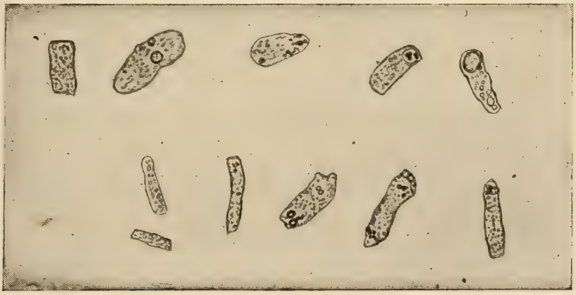

Fig. 64.—Cells which resemble casts found in fluid massaged from a prostate, the seat of chronic prostatis. (Kindness of Dr. George Walker, of Baltimore, who will later publish this and similar cases.)

I ^{5} will contain the exudate in the posterior urethra which will usually be washed clean by the flow of urine.

I ^{6} may contain the last traces from the posterior urethra, and

I ^{7} will contain also urine from the most dependent portions of the bladder, also the contents of the prostatic and ejaculatory ducts which often do not discharge the exudate collected in them until the muscular contractions made at the end of micturition force out the plugs of thickened exudate which occlude their mouths (Fürbinger's hooks).

MICRO-ORGANISMS OF THE EXTERNAL GENITALIA

Bacillus Ulceris Cancrosi (Ducrey's Bacillus).—This organism is now recognized as the cause of soft chancre. It is found in smears of the purulent discharge from these sores, but always mixed with a host of other organisms. (Sections of the tissue show it in pure culture.)

This bacillus is a small oval rod about 1.5μ long and 0.5μ thick, which stains readily in all bacterial stains, but decolorizes very easily. It is a very poor grower indeed but some claim it can be cultivated on blood agar.

Treponema Pallidum, Spirocheta Pallidum.—This organism (see Fig. 65) is a spirocheta the average length of which is from 6 to 15μ (although some are even 20μ long), and thickness 0.25μ. Noguchi[169] described 3 strains which differ in coarseness: the thicker, 0.3μ in width; the thinner, 0.2μ, and the average, 0.25μ. It is tightly twisted like a corkscrew in 5 or 6 or more regular, closely set and rigid curves, the fineness and regularity of which is a characteristic feature. It is pointed at each end. It is a flagellated organism, but shows only a twisting, rotating, or bending motion. They are usually found singly, although sometimes several are tangled.

To secure a good specimen when searching for Treponema pallidum one should choose a lesion which is likely to be rich in these organisms, as the suspected chancre, mucous patches, tonsils, condylomata and enlarged glands. It is also possible to obtain them from a fresh skin eruption. In the case of superficial lesions the serum should be obtained from the deeper layers of tissue, since this usually is richer in treponema and freer from contamination than is that from nearer the surface. Among the contaminating spirochetæ are: in the mouth, Spirocheta bucallis, Treponema microdentium and macrodentium, Treponema mucosum, and the Spirillum of Vincent; on, or near the genitals, Spirocheta refringens and Treponema calligyrum.

The examination of sores and ulcers which may be chancres is especially important because the Wassermann test at this stage of lues may be negative.

The sore to be examined is first washed with sterile physiological salt solution, then its superficial tissue is removed by means of a sharp, sterile curet. The blood is wiped away with a piece of clean gauze. After the bleeding lessens and the blood becomes more serous, the sore is squeezed by the fingers and the drop of blood which is pressed from the deeper tissues is caught on a clean thin slide and at once covered with a cover-glass if the fresh specimen is to be examined with dark-field illumination. The cover-glass is gently pressed down to secure a thin, even layer of serum, drops of cedar oil are applied to the cover-glass and another opposite this on the slide and the specimen then fitted onto the stage of a microscope supplied with attachments for dark-field work. The light must be carefully manipulated or the thin, delicate treponema may be overlooked. This method of examination is the surest as well as the quickest and easiest. The staining methods are slow and less reliable. In primary and secondary lues this is a safer method than the serum reaction.

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[169] Jour. Exp. Med., 1912, vol. xv, p. 201.

If a medicated dusting powder, an ointment, or a wash has been used on the lesion, the search usually is fruitless. In that case the lesion should be washed for several days with plain soap and water and then the examination made.

Enlarged lymph-glands may be carefully aspirated with a fine needle on a tightly fitting glass syringe.

In examining mouth lesions great care should be taken to obtain the serum from the deeper layers of tissue, since so many other spirochetæ may be present.

In the examination of skin lesions the superficial layer of epidermis is removed by means of a sharp curet and the serum squeezed from the cutis.

To find spirochetæ similar in appearance from quite different parts of the body will often clear up a doubtful diagnosis.

If blood is to be examined, 1 c.c. of blood is mixed with 10 c.c. of 0.3% acetic acid, this fluid is then centrifugalized and the sediment examined.

If stained specimens are desired very thin smears should be made. This organism is stained with great difficulty.

The *Giemsa staining mixture* in common use is:

Azur II eosin, 3 gms.;
Azur II, 0.8 gm.;
Glycerin (Merck C. P.), 250 gms.;
Methyl alcohol (Kahlbaum I), 250 gms.

The specimen, dried in the air and then fixed for 1 hour in absolute alcohol, is stained for 24 hours in a fresh dilution of this stain (1 drop of the above mixture to 1 c.c. of distilled water). The specimen is then examined. If the nuclei of the leucocytes have taken a deep blackish red color the smear is well enough stained to justify the long search necessary to find the organisms, which take a delicate violet-purple color.

Cultivation of Treponema Pallidum.[170]—A piece of sterile fresh rabbit's kidney or testicle is placed at the bottom of each of 6 tubes which measure 20 cm. in length and 2 cm. in width and then into each tube is poured 15 c.c. of mixture consisting of 2 parts of 2% agar, slightly alkaline and heated to 50° C., and 1 part of ascitic or hydrocele fluid. After this solidifies a layer of sterile paraffine oil 3 cm. deep is superimposed.

The tissue from which cultures are to be made is first cleansed with sterile salt solution and then suitable fragments are snipped off. These are immediately immersed in sterile salt solution containing 1% sodium citrate. These fragments of tissue, which should be carefully preserved against drying, are then cut into small bits. One such bit is emulsified in a mortar with the citrate solution and this emulsion examined under the dark-field microscope to determine definitely that spirochetæ are present. The other bits of tissue are used to inoculate the tubes of media. One of these particles is forced to the bottom of each culture tube by means of a blunt glass rod or heavy platinum loop, and into the same tube several drops of the emulsion are introduced deeply by a capillary pipet, care being taken to avoid tearing the medium.

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[170] Noguchi, Jour. of Exp. Med., 1911, xiv, p. 99 and 1912, xv, p. 90.

These tubes are inoculated at 37° C. continuously for 2 or 3 weeks before examining them. By that time they usually show a dense opaque growth of bacteria along the stab-canal and the diffuse opalescence of spirochetæ growth radiating from this. The solid medium is now pierced by a capillary pipet to obtain some of this latter growth which is examined under the dark-field. A mixed culture is generally found. Fresh tubes are now inoculated with material obtained with a capillary pipet, which is introduced deeply into the agar column even to the bottom of the tube, without striking the central stab-canal. The contents of the pipet are expressed by means of compressed air, as, for instance with a syringe. These tubes are inoculated for 2 or 3 weeks at 37° C. and are again inspected. If the faint hazy zone around the stab-canal (and due to the growth of spirochetæ) has extended far enough to allow one to penetrate this zone with a capillary pipet one makes another transfer as follows:

The surface of the medium is sterilized with sublimate alcohol. The tube is now scratched about its middle with a diamond pencil and the glass cracked by a red-hot glass rod which is touched to the scratch. The upper half of the test-tube is then removed. The exposed surface of the agar is sterilized with sublimate alcohol, after which all remaining moisture is wiped away with sterile absorbent gauze. The agar column is now bent gently until it cracks transversely thus exposing a clear surface on which the hazy growth is readily seen. Now, without touching the central canal, a capillary pipet is introduced into this haze and, after confirming by dark-field examination the presence of pallidum, a fresh series of inoculations is made. Several reinoculations are as a rule necessary before a pure culture is obtained.

Treponema pallidum is very anërobic and migrates into the solid media from the inoculation stab. This organism can be grown in liquid media.[171]

Spirocheta Refringens (see Fig. 65, *d*).—Spirocheta refringens is often found together with Spirocheta pallidum. It is a common parasite occurring in great numbers in many ulcerative lesions. It is larger, thicker and more refractile than Treponema pallidum. Its spirals are broader, more wavy and more irregular, its ends are blunter, it occurs in greater numbers in a smear and it stains more easily than does pallidum.

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[171] Noguchi, Jour. of Exp. Med., 1912, xvi, p. 211.

Treponema Microdentium.—Noguchi[172] was the first to isolate Treponema microdentium in pure culture from the mouth. It is obtained from tooth deposit, preferably from a young child, and grown in much the same manner as is Treponema pallidum. It is, however, a much more rapid grower. This organism measures less than 0.25μ in width at the middle of the body and gradually tapers towards both extremities, which are sharply pointed. Organisms from old cultures may reach 8μ in length and show, on an average, 14 curves. The ends are usually drawn out straight. It is non-pathogenic. The cultures have a characteristic putrefactive odor, not, however, that of pyorrhea alveolaris.

Treponema Macrodentium.—Noguchi[173] found Treponema macrodentium most frequently in the mucus about the tonsils and pharynx and in large numbers in the exudate in ulcerative stomatitis. Its cultivation is more tedious than that of microdentium. Its morphology differs considerably according to the age of the cultures. Young organisms are plump and short with from 2 to 8 rather irregular shallow curves. Its width varies from 0.7 to 1.0μ, and its length from 3 to 8μ. It is doubly refractile. Its cultures have no odor.

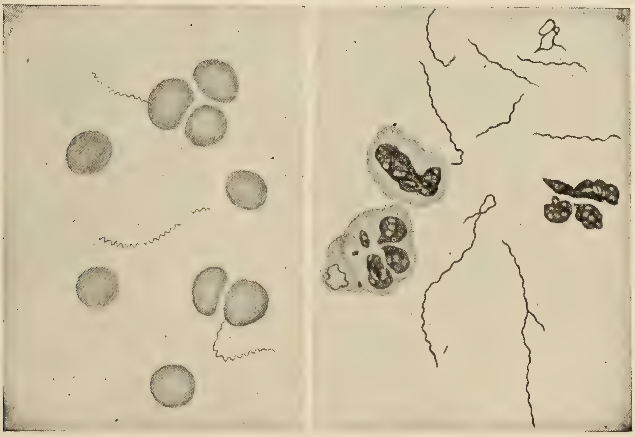

Fig. 65.—On the left Treponema pallidum (Spirocheta pallidum). A smear from a chancre. On the right Spirocheta refringens. A smear from a chancroid.

Treponema Mucosum.—Noguchi[174] isolated Treponema mucosum from the pus around the roots of the teeth of a case of pyorrhea alveolaris. Its most striking features are its capacity to produce in pure culture a mucin and a strong fetid odor. The pus was gathered from the gum margin in a sterile capillary pipet and suspended in a few cubic centimeters of sterile citrate solution. Tubes of medium similar to that used for Treponema pallidum are innoculated with this suspension culture.

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[172] Jour. of Exp. Med., 1912, xv, p. 81

[173] Ibid.

[174] Jour. Exp. Med., 1912, xvi p. 194.

These organisms measure from 0.25 to 0.3μ in width, and from 8 to 12μ in length. The curves number from 6 to 8, are remarkably regular and often quite deep. Both extremities are sharply pointed. It cannot be distinguished from Treponema pallidum by its morphology alone, but can by cultivation. This organism explains in part at least the characteristic odor of the breath in pyorrhea alveolaris.

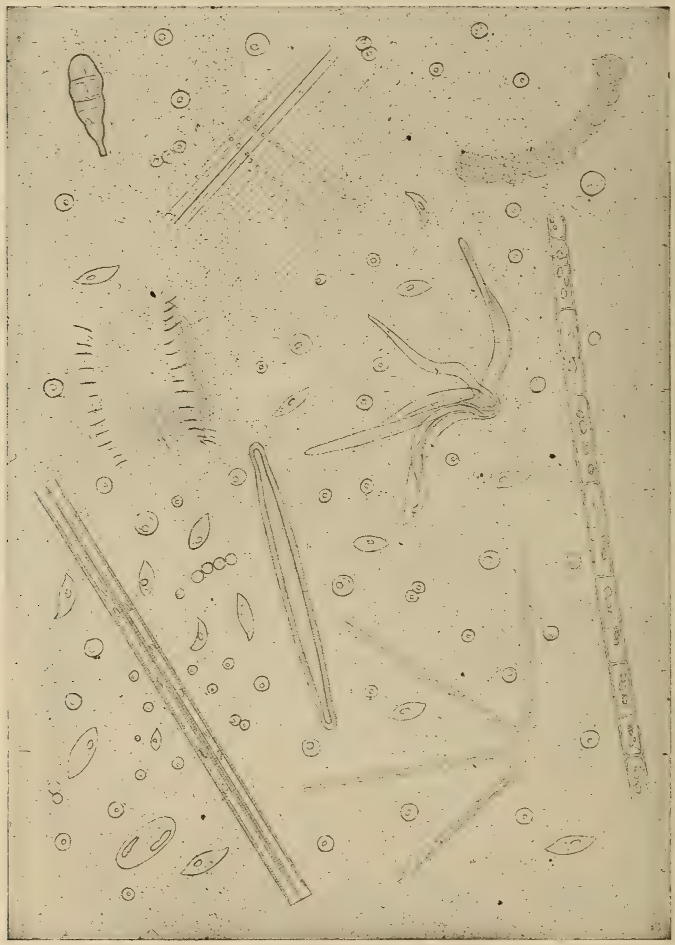

FIG. 66.—Protophytes and other low forms of life often found in tap water. × 400.

TREPONEMA CALLIGYRUM.[175]—Treponema calligyrum is an organism found by Noguchi on the surface of genital and anal lesions of luetic and non-luetic patients. It is non-pathogenic. Morphologically it stands between Treponema pallidum and Spirocheta refringens. It is easily cultivated.

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[175] Noguchi, Jour. of Exp. Med., Jan. 1, 1913.

On the skin about the genitalia is an abundant flora of organisms: streptococci, staphylococci (especially Staphylococcus albus), Bacillus coli communis, Bacillus pyocyaneus, Bacillus lactis aërogenes, Bacillus aërogenes capsulatus, and various strains of smegma bacilli.

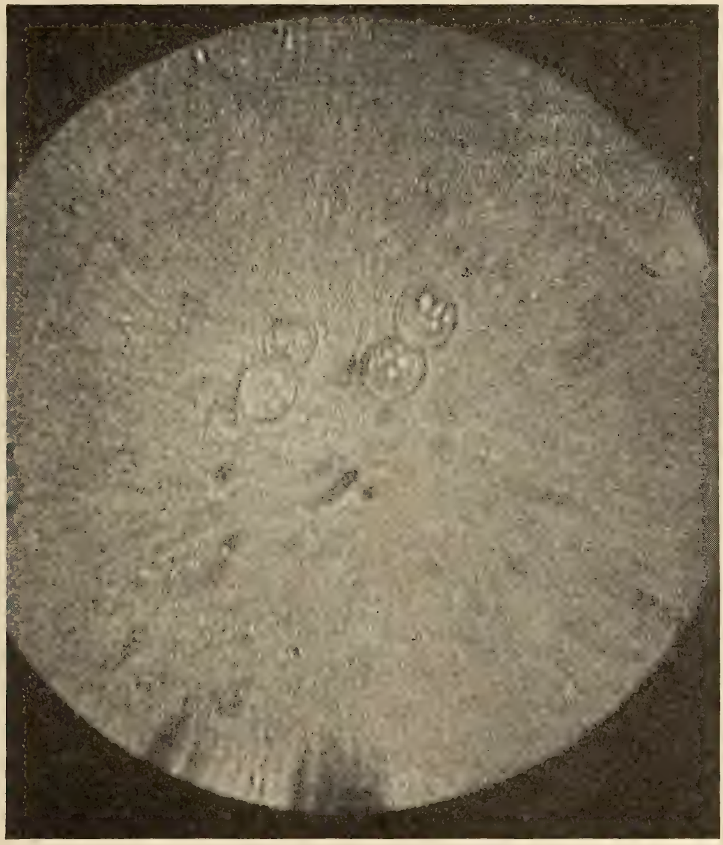

FIG. 67.—Blastomycetes in the urine.

YEASTS AND MOULDS IN URINE

The protophytes added from tap water should be recognized (Fig. 66).

Yeasts.—Ordinary yeast cells from the air or surface of the body often reach the urine and sometimes multiply so abundantly that they are conspicuous in the sediment, especially in cases of diabetes. They may gain entrance to the bladder and there ferment the sugar before the urine is voided thus giving rise to " pneumaturia." If they are to be cultivated the urine should be kept acid with acetic acid.

In cases of systemic blastomycosis this organism may be found in the urine in large numbers (see Fig. 67).

Moulds may occasionally be found in fresh urine but the most are later contaminations. In one case of pyelitis, previously treated by repeated irrigations through ureteral catheters, the urine on one occasion was very bloody, and contained masses of the mycelium of some mould which would not grow on the various media tried. One thorough irrigation cleared up this infection. The chances are that at a previous catheterization some spores were introduced into the pelvis of the kidney.

Sarcinæ, smaller than those found in the gastric contents, may also be found in the urine.

ANIMAL PARASITES

Among the animal parasites which may be demonstrated in the urine are the hooklets, daughter cysts (even several hundred in a case) and fragments of membrane of echinococcus cysts. There will be no urinary symptoms of hydatid disease of the kidney, unless perhaps a catarrhal pyelitis, unless the cyst ruptures into the urinary tract and then the urine will appear watery, soapy, or bloody. Embryos of filaria are found in the urine in cases of tropical hematochyluria (see page 209).

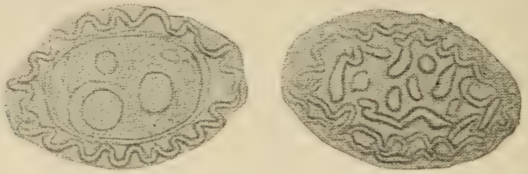

Fig. 68.—Eggs of Eustrongylus gigas. × 400.

Flagellates belonging to the cercomonas or trichomonas groups often are found in the urine. There is a dispute whether they have ever been found in a fresh catheterized specimen. Many may be later contaminations. *Balantidium coli* however would seem to infect the urinary tract and be found in the freshly voided urine, from 5 to 15 parasites in each field under the microscope, as in Hinkelmann's [176] case.

Eustrongylus Gigas.—A few cases of Eustrongylus gigas infection have been reported, but in most of them there certainly was a mistake in diagnosis. In 1 case of chyluria, however, the eggs were found [177] (see Fig. 68).

Schistosoma Hematobium (Bilharzia).—The trematode worm, Schistosoma hematobium (see Fig. 69), so common in Africa, especially in Egypt and the Transvaal, has been found twice in Porto Rico (Martinez) and six times in this country.[178]

The male measures from 12 to 14 mm. in length, is flat but so folded that it appears cylindrical and forms a gynecophoric canal which receives the female. The female is 20 mm. long and is filiform. The adults live in the portal vein, also its branches and in other veins of the abdomen and pelvis, especially those of the bladder, the pelvis of the kidney and the rectum. The eggs (Figs. 102 and 103) are large, from 120 to 190μ long and from 50 to 73μ wide, are fusiform, have no operculum and have a spine which may be terminal or lateral (Schistosoma mansoni?).

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[176] New York Med. Jour., Jan. 30, 1915.

[177] Stuertz, Deutsches Arch. f. klin. Med., 1903, vol. lxxviii, p. 586.

[178] See O'Neil, Boston Med. and Surg. Jour., October 27, 1904, vol. cli, p. 453, also Dr. Daywalt's letter in Dr. Arnold's paper, Southern Practitioner, 1906, vol. xxviii, p. 13.

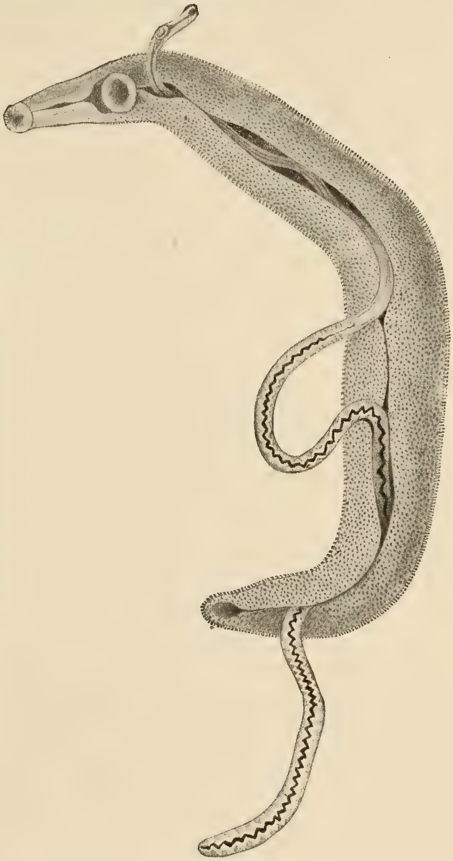

FIG. 69.—Schistosoma hematobium, adult worms. (Copied from Braun.)

The urinary symptoms of this infection are catarrh of the bladder and hemorrhages ("Egyptian hematuria"). At first only blood flecks are passed at the end of micturition but later the hemorrhages may be profuse. The symptoms are caused by the eggs which the female deposits in the mucous membrane. These may be passed with the urine, but any, especially those with a lateral spine, remain in the bladder and form the nucleus of calculi. Each egg contains a miracidium which is completely ciliated. The shell splits when the urine is diluted with water. What the intermediary host is, and how human infection occurs, are not known.

Nematode worms other than filaria are sometimes found in the urine, especially Anguillula aceti, or the "vinegar eel." Stiles reports one case of infection of the bladder with this worm. Other cases may be due to contamination from the bottle in which the urine is collected.[179] These worms resemble closely Strongyloides intestinalis, except that they (A. aceti) are slightly longer (males 1.2 mm. long and 0.033 mm. wide; females 1.9 mm. long and 0.06 mm. wide; embryos 0.25 to 0.3 mm. by 0.015 mm.).

The student should always be able to recognize the various plant contaminations which occur in tap and stagnant water and in vessels rinsed out with this water. That is, he should be able to recognize that they are of no significance (see Fig. 66).

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[179] Billings and Miller, American Medicine, May 31, 1902.

PROSTATIC FLUID

The prostatic fluid is best obtained by "milking" this gland by the finger in the rectum. The urethra is first well washed, then the fluid thus expressed and collected. The amount under normal conditions varies greatly, from none to even 5 c.c. at 1 milking. It has a grayish-white, yellow, or greenish color, a milky turbidity due to lecithin globules and a characteristic odor. It is slightly viscid, tenacious, of low specific gravity and contains but little solid matter (from 1 to 2%). The reaction of the prostatic fluid has attracted considerable attention because of the possibility that this may be an important factor in the production of sterility. Thus far no results of importance have been gained. It reacts faintly alkaline to most reagents and acid to others, but this varies much.

One examines this fluid for motile spermatozoa immediately on obtaining it, then adds a drop of acetic acid to bring out the cells more clearly and examines it for pus-cells (Fig. 61, *b*).

Microscopically, the most striking objects in prostatic fluids are the great numbers of lecithin globules (Fig. 61, *a*) which give it its milky appearance. These vary in size from those very minute to others even half the size of a red blood-cell. They are not very refractive and so can be distinguished readily from fat. An increase in their numbers in the fluid of patients with chronic prostatis indicates improvement. Corpora amylacea (Fig. 61, *d*; 62, *c*) are sometimes found in the urine, especially of elderly men. They have no significance. They are laminated and have a finely granular center. Of their composition nothing is known except that they stain blue with iodine. Epithelial cells of various kinds are present. Some are large and polygonal, others are cylindrical; they are found single or in groups, and vary much in size (see Figs. 61, *c*; 62, *a*). Interesting cell-like masses which vary much in size, the so-called granular cells (Fig. 62, *e*), which may resemble colostrum corpuscles, are merely masses of fat-like granules. These break down and liberate the refractive globules seen free in the fluid (Fig. 61, *e*). Some of the granules resemble myelin (Fig. 62, *d*). Columnar epithelial cells and cylindrical cells which resemble casts (see Fig. 64) are sometimes present. One finds also large clear cells of varying size, with or without a nucleus (see Fig. 62, *b*), which are supposed also to arise in the seminal vesicles. In normal prostatic fluid one finds no pus-cells and no red blood-corpuscles. Spermatozoa (Fig. 61, *f*) are usually present in prostatic fluid.[180]

Prostatic fluid should be examined for spermatozoa while it is as fresh as possible since it is important if possible to see them move. To study their finer structure the fluid, if much proteid is present, should be diluted with even 20 volumes of water and smears made which are dried in the air, heated to 120° and cooled slowly. The specimen is then covered with

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[180] For a description of these, see University of Pennsylvania Med. Bull., No. 3, 1902.

2% iron-alum solution for from 2 to 4 hours, washed in water and then with 1% hematoxylin for 12 hours. It is then decolorized carefully with 1% iron-alum, counterstained for from 1 to 3 minutes with a saturated aqueous solution of eosin, dried and mounted. Many of the spermatozoa are abnormal in shape. Some have 2 heads and some even 3 tails. These monsters are never seen to move. One seldom tries to determine more than their presence and the motility. If they are motile, one concludes that they are functionally normal. If they are not found or are not moving no conclusions at all are justified.

In fluids from cases of acute or chronic prostatitis many leucocytes are present and the lecithin globules are diminished in number.

Spermin crystals may be demonstrated by adding to the prostatic fluid 1 drop of 1% ammonium phosphate solution and allowing the specimen to dry for 2 hours under the cover-glass. These crystals resemble somewhat the Charcot-Leyden crystals. They are colorless, transparent needles or whetstones, many of which are imperfectly crystalized.

Prostatic casts or testicular casts (see page 273).

Gonorrheal threads are described on page 296.

The short coma-like flocculi which are sometimes seen, form in the excretory ducts of the urethral glands and follicles and mean an intense involvement of these structures. Those found in the second glass are from the prostatic glands and are signs of chronic prostatitis. They consist of superimposed layers of cylindrical epithelium.

Prostatic plugs, which are large cylindrical masses of mucus, are sometimes seen. These form in mild inflammations of the prostatic ducts. Some of these mucous masses are full of spermatozoa (Fig. 63).

FUNCTIONAL RENAL DIAGNOSIS

Methods of estimating with some degree of accuracy the functional ability of various organs and especially of the kidneys is one of the best recent contributions to medicine. The discrepancies between the kidneys as found at autopsy and that which one would expect from the urinary examinations alone are proverbial. Small contracted kidneys, less than ½ or ⅓ the normal size, may excrete a urine normal in amount and in specific gravity, which contains but a trace of albumin and very few casts; in other cases with marked albuminuria and cylindruria no clear evidence of nephritis may be found; while some patients have died in so-called uremia who had little clinical evidence of renal insufficiency, whose urine contained but a trace of albumin and whose kidneys at autopsy showed slight changes. Evidently the time-honored clinical, chemical and microscopical methods are no test of the important lesions. They may indicate the severity of the acute lesion then in progress, but they certainly do not test the amount of renal substance left by disease, nor the ability of this to do its work (see page 323). Surely there must be some methods of predicting an imminent death from renal insufficiency, some means of foreseeing an on-coming uremia. These means may be found in functional renal diagnosis and in blood chemistry.

The problem of functional renal diagnosis is not so much to determine the anatomical condition of the kidney as its ability to do a stated amount of work in a given time. A well-compensated severe lesion is manifestly of less immediate danger than a poorly or non-compensated acute lesion.

Before describing the tests for renal functional ability it is well to state that the toxins so evident from their results in nephritis are themselves as yet unknown unless it be that they are the same bacteria which produce infections elsewhere in the body. We believe this to be true and yet the kidney function is not reduced in every general infection nor yet in many, as a study of the general septicemias shows, while in many cases of severe or fatal nephritis the evidence of general infections may be unsatisfactory. It is easy to assume that the kidney must be a relatively difficult organ to infect since its function is to remove all poisons from the body and this would include the toxin of infections. It is also easy to assume that a case of nephritis is chronic because its cause is chronic. At least we can say that there is little evidence of the vicious circle formerly so emphasized which was supposed to keep a nephritis in progress, for once clean out the infections elsewhere in the body and in some cases the nephritis at once begins to subside. The function tests impose upon the kidneys a definite task and measure their success in fulfilling it. From their success in eliminating a given amount of some substance in a given time we by analogy surmise how well they perform their other functions. At the onset it should be confessed that the functions of the kidney are not as well understood by the physiologist as the clinician seems to assume when he uses the methylene-blue test to test the " epithelial filter," the salicylic acid test to test the "glomerular filter" and phlorizin to test the "glandular activity " of the renal epithelium.

No one test is above criticism; one is never enough and several must be used to get a fair renal picture.

We wish to warn workers that these functional tests make an unusual demand on the kindeys and one to which they may not be able to respond, so that slight reactions do follow and disastrous results may follow even the simplest.

Physiochemical Tests—CRYOSCOPY, FREEZING POINT OF THE URINE.—Cryoscopy, or the determination of the depression of the freezing point of water by a salt in solution, is a well recognized and very valuable method of physical chemistry to determine the molecular weight of a substance and the degree of disassociation of its molecules. Clinical chemists attempted (by determining the freezing point of urine and of the plasma) to estimate how many products of katabolism the kidneys have excreted in the urine and how many are still in the plasma. But even when the problems are of the simplest nature, *i.e.*, when dilute solutions of a single and pure salt are used, this method requires experience, skill and due regard to a good many factors which can modify the results. It is hard to see, therefore, how this method could be applied with much success to complex fluids like the urine or blood, in which are dissolved a great variety of bodies of widely different nature, some unknown; and yet most definite conclusions concerning the functional ability of the kidney were drawn from changes in the freezing point of these fluids which a physical chemist would consider slight.

ELECTRICAL CONDUCTIVITY.—This excellent method of physical chemistry also was appropriated by the clinician in his desire to estimate the functional ability of the kidneys. By electrical conductivity is meant the reciprocal of the resistance which a certain amount of a solution between 2 platinum electrodes of given size and given distance apart offers to the passage of a current of known strength. This is really a measure of the number of electrolytes in solution, that is, of the disassociated ions. It is not affected by such bodies as albumin, sugar, urea, which are not disassociated, and hence is practically a measure of a few salts in the blood and urine, especially the chlorides. It is difficult enough to get accurate results using a simple dilute solution of one salt and it certainly is working in darkness to apply this very delicate method to the blood and urine, which contain an unknown mixture of various bodies. It therefore is little wonder that this method gave nothing definite.

Methods Using Normal Products of Metabolism.—The time of excretion of urea is an old criterion of functional renal ability. In acute nephritis it may take 3 to 6 days to excrete the urea resulting from 1 day's meals; in chronic nephritis and renal tuberculosis, 2 days. One therefore gives the patient a meal of measured ingredients and then follows the curve of the elimination of urea. During this delay the urea accumulates in part in the blood and may be determined there quantitatively (see page 542). When it is increased tenfold (*i.e.*, to 0.3%) there is danger of uremia (Herter).

THE SALT AND WATER TEST.—The patient is kept for a few days on a diet constant in water (about 2000 c.c. per day) and in chlorides (about 5 gms. per day, all the chlorides of foods included). After 2 days on which the chloride output has been fairly constant the patient is given at 1 time from 5 to 10 gms. of salt and the curve of its elimination determined.

Normally, an increased salt intake is eliminated in 1 of 2 ways, depending on the amount of water given at the same time. If given without increasing the water intake it is excreted within 24 hours and without any diuresis. If extra water was given at the same time its excretion is accomplished partly by increasing the concentration of the urine and partly by diuresis. The excretion of salt is by the tubules (Schlayer).

In cases with moderately severe vascular injury of the kidney the administration of the salt may be followed by a marked diuresis and all the salt be excreted within 24 hours, but without any increase in the concentration of the urine (*i.e.*, its specific gravity remains rather low and fairly constant). This condition (vascular hyposthenuria of Schlayer) seems due not so much to a lesion of the tubules as to hypersensitiveness of the vessels. If, however, the vascular lesion is severe the kidney responds to the administration of the salt by an oliguria.

In cases with severe tubular destruction the urine is little affected by the dose of extra sodium chloride, since the tubules are unable to excrete it (tubular hyposthenuria).

THE DILUTION TEST.—The excretion of water is so important a function of the kidney that it is natural to assume that variations in this would be a very simple and satisfactory test of renal permeability. This test is applied in a simple form as follows: The patient drinks an unusual amount of water with his evening meal and practically none until the following morning. He voids on retiring, which should be at least 4 hours after the meal, and again in the morning. A normal person will eliminate the most of the excess of fluid in the evening specimen which will therefore have a lower specific gravity than the morning specimen. If the specific gravity of the morning specimen is lower than that of the evening there is evidence of vascular renal lesion; but if the 2 are almost equal this " fixation of specific gravity " indicates a severe grade of renal insufficiency.

In a more elaborate way this test may be performed as follows: the specific gravity of the patient's urine is determined and then he is asked to drink from 1 to 2 liters of water. The time of the appearance and the duration of the resulting polyuria and of the lowering of the specific gravity are noted. To determine the combined efficiency of the kidneys each half hour's voiding is examined separately but to determine the relative efficiency of the 2 kidneys the ureteral catheters are introduced and left in place for at least 3 hours and the urine from each is collected in half-hourly portions.

The dilution of the urine may begin during the second half hour, may reach its maximum in 2 or 3 hours and last 5 or 6 hours. In parenchymatous nephritis the ability to eliminate an excess of water seems more reduced than in cases of contracted kidney. If the kidneys are not equally diseased the excretion of the diseased one will be more uniform than that of the more normal organ; that is, the more normal kidney will show the dilution first and more markedly that the other.

The test thus refined has little value. Even in the normal person we find no uniformity in the time of the appearance of, or in the duration of, a polyuria following the ingestion of large amounts of water. Again, there may already be a polyuria of the diseased side which will mask any increase of the output of the more normal side. And, lastly, 3 hours is a long time to allow ureteral catheters to remain in position.

TEST MEALS.—Several test-meals have been proposed to test renal function. That is, the patient consumes weighed meals selected with a view to taxing somewhat the renal function and the urine is studied to ascertain the ability of the kidneys to meet the problem.

Hedinger and Schlayer had proposed as an improvement over v. Monakow's "added urea and salt test," which requires 10 or 12 or even more days, a 2-hour renal test which could be completed, so far as the patient is concerned, in 24 hours. The results are very much the same. *Mosenthal*[181] proposed the modification of their diet described below. All food is to be salt-free food from the diet kitchen. Salt for each meal will be furnished in weighed amounts. All food or fluid not taken must be weighed or measured after meals. No other food or fluid of any kind is allowed.

- Breakfast, 8 A.M.:
  - Boiled oatmeal 100 gms.
  - Sugar 1–2 teaspoonfuls.
  - Milk 30 c.c.
  - Two slices of bread (30 gms. each).
  - Butter 20 gms.
  - Coffee 160 c.c.
  - Sugar 1 teaspoonful } 200 c.c.
  - Milk 40 c.c.
  - Milk 200 c.c.
  - Water 200 c.c.
- Dinner, 12 NOON:
  - Meat soup 180 c.c.
  - Beafsteak 100 gms.
  - Potato (baked, mashed or boiled) 130 gms.
  - Green vegetables as desired.
  - Two slices of bread (30 gms. each).
  - Butter 20 gms.
  - Tea 180 c.c.
  - Sugar 1 teaspoonful } 200 c.c.
  - Milk 20 c.c.
  - Water 250 c.c.
  - Pudding (tapioca or rice) 110 gms.
- Supper, 5 P.M.:
  - Two eggs cooked in any style.
  - Two slices bread (30 gms. each).
  - Butter 20 gms.
  - Tea 180 c.c.
  - Sugar 1 teaspoonful } 200 c.c.
  - Milk 20 c.c.
  - Fruit (stewed or fresh) 1 portion.
  - Water 300 c.c.

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[181] Arch. of Int. Med., 1915, xvi, p. 733.

No food or fluid is to be given during the preceding night or until 8 o'clock the next morning (after voiding), when the regular diet is resumed.

The patient empties his bladder at 8 A.M. and then each 2 hours until 8 P.M. The urine from 8 P.M. to 8 A.M. is collected in 1 specimen.

The above diet contains approximately 13.4 gms. of nitrogen, 8.5 gms. of salt, 1760 c.c. of fluid and a considerable quantity of purin material in the meat, soup, tea and coffee which act as diuretics.

O'Hare's Modification of Hedinger and Schlayer's " Two-hour Test.[182]

O'Hare, the next year, proposed the following menu for the test day:

Seven A.M.: coffee, milk, sugar, toast, and butter; 10 A.M.: milk, toast and butter; 12.30 P.M.: bouillon, broiled steak, butter, mashed potato, butter, toast and butter, coffee, milk and sugar; 4 P.M.: tea, milk, sugar, crackers; 7 P.M.: soft egg, blanc mange (1 egg, sugar, cornstarch, milk) and cream. Amounts sufficient to give approximately 2500 calories, 1550 c.c. of fluid, 76 gms. of protein, 127 gms. of fat, 245 gms. of carbohydrates and 5.8 gms. of sodium chloride.

This is a mixed diet containing food diuretics of various types (purins, salts, water, etc.). Each of the 5 unequal portions contain known but varying amounts of fluid, nitrogen and salt—the noon meal the most of all.

On the 2 days preceding this day the patient should receive about 2000 calories, 75 gms. of protein and 4 gms. of sodium chloride. The urine is collected each 2 hours from 7 A.M. to 9 P.M. and then 1 " night specimen " from 9 P.M. and 7 A.M. Each specimen is analyzed for volume, specific gravity, total nitrogen concentration, total chloride and chloride concentration and the results are charted.

The purpose of these tests is to find out to what extent and in what manner the diseased kidney under stimulation by the different diuretics taken in the food reacts by putting out the varying amounts of water, nitrogen and chloride ingested. Normal cases respond by putting out water and salt promptly and in good amounts. The diseased kidney, however, may show that its power to excrete is too low to accommodate the large amounts of these elements ingested, especially at the noon meal. These " fixed " cases show a curve of excretion that approaches more or less a straight line. Instead of getting the normal " picket fence " curves we find some or all of the curves of excretion more or less flattened out.

In general the salt excretion is impaired before there is much disturbance of water and nitrogen excretion; in most patients salt and water excretion behave very similarly; the nitrogen excretion is greatly impaired only in the severe cases. Salt, water and nitrogen excretion, however, show some disturbance in mild cases in which the phenolsulphonephthalein test is normal and there is no increased blood nitrogen. These dietary tests cannot be used in very severe cases of chronic nephritis.

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[182] Arch. of Int. Med., June, 1916, xvii, p. 711.

The specific gravity (quoted from Mosenthal) of the different voidings of a normal individual varies 10 points or more from the highest to the lowest. The night urine has a high specific gravity (1.018 or more), a high percentage of nitrogen (above 1%) and is small in amount (400 c.c. or less). The quantities of water, salt and nitrogen excreted approximate the intake. When kidney function is lowered the first signs are usually demonstrated in the night urine; its quantity increases while its specific gravity and nitrogen concentration are lowered. One or all of these departures from the normal may occur. In severe cases of chronic nephritis the functional renal inadequacy is indicated by a markedly fixed and low specific gravity, a diminished output of both salt and nitrogen, a tendency to total polyuria and a night urine showing an increased volume, a low specific gravity and a low nitrogen concentration. Such functional pictures, however, are not confined to nephritis, but are found regularly in many other conditions; pyelitis, cystitis, hypertrophied prostate, marked anemia, pyelonephritis, polycystic kidney and diabetes insipidus.

The causes of diminished renal function must be sought for in the urinary passages, in the blood or in the kidney itself. Prognosis and therapy will depend largely on the cause of the fundamental impairment and not on its degree. A divergence between the degree of functional renal involvement and the intensity of the signs and symptoms of nephritis is frequently found and accentuate the lack of parallelism that there may be between functional and anatomical lesion.

In chronic diffuse (parenchymatous) nephritis the condition of renal function is characterized by its variability. In these cases the results of the test-meal have proved to be extremely valuable in giving an idea of the status of salt, nitrogen and water excretion, as well as a picture of renal efficiency as a whole. The findings in myocardial insufficiency vary according to the condition of the heart. They differ much in the periods when there is myocardial decompensation and the accumulation of edema, when the edema is subsiding and later when cardiac compensation is again fully established. In such cases it requires some time before the kidney resumes its normal activity. This intervening period before the function is normal is indicated by a tendency to a low, fixed specific gravity and a nocturnal polyuria. During the period of full myocardial decompensation the results of kidney activity are very characteristic; the specific gravity is markedly fixed at the level of about 1.020, the salt output is diminished, that of nitrogen is high in marked contrast to the salt and there is oliguria. When chronic nephritis and cardiac decomposition coexist, as they so often do in hypertensive nephritis, the urine may exhibit the characteristics of either lesion. The determining factor is probably the chronic nephritis which may or may not be so far advanced as to present an unchanging barrier to the influence of renal congestion.

Ambard's Coefficient.[183]—Ambard was one of the first to show that the excretion of urea, sodium chloride, etc., is carried on by the kidneys according to definite laws capable of numerical expression. In the case of urea Ambard's formula, known as Ambard's coefficient, is as follows:

$$\frac{\mathrm{Ur}}{\sqrt{\mathrm{D}\times\frac{70}{\mathrm{Wt}}\times\sqrt{\frac{\mathrm{C}}{25}}}}=\mathrm{C}.$$

Ur = grams of urea per 1 liter of blood;
D = grams of urea excreted per 24 hours;
C = grams of urea per 1 liter of urine;
Wt = weight of patient in kilos;

70 (kilos) is accepted as a standard weight and 25 (grams per liter) as a standard concentration of urea.

The value obtained for C in normal subjects lies between 0.060 and 0.080, the mean about 0.080.

Changes in C indicate changes in the blood-urea, which changes vary as the square root of changes in the rate of excretion. In order that pathological variations in rate of excretion may be expressed according to a scale of 100 (so that *e.g.*, if the index were 50 it would indicate that the condition was 50% of normal), and granting that Ambard's coefficient of 0.080 is the mean normal index, the formula according to McLean,[184] may be written as follows:

$$\text{Index}=\frac{\text{grams of urea per 24 hours }\sqrt{\text{grams of urea 1 L. of urine x 8.96}}}{\text{Wt in kilos}\times(\text{grams of urea per 1 L. of blood})}$$

Ambard's coefficient for chloride excretion. This formula as given by McLean is:

$$\text{Plasma NaCl}=5.62+\sqrt{\frac{\text{Grams NaCl per 24 hrs. }\sqrt{\text{grams per 1 L. of urine}}}{4.23\times\text{Wt in kilos}}}$$

In this formula 5.62 is the normal threshold limit experimentally determined of NaCl in the plasma (*e.g.* no NaCl will be eliminated in the urine unless that in the blood exceeds 5.62 gms. per 1 L. of plasma.

Normal Excretion of Urea and Sodium Chloride (McLean)—*Collection of Specimens.*—Short periods, *e.g.*, of 72 minutes, during either the forenoon or afternoon, are preferable for observation of the excretion of urea and sodium chloride. By collecting the urine of a given period and withdrawing the blood at the middle of this period the blood sample may be assumed to represent the average for that period. If no food or water is taken during the period, and this begins not too soon after a heavy meal, the rate of excretion during the period will remain practically constant. One-half hour before the test begins the patient drinks 150 to 200 c.c. of water, and takes no more fluid or food until the end of the observation. At the beginning of the period the bladder is emptied; 36 minutes later about 10 c.c. of blood are taken from an arm vein into a dry tube containing about 100 mgms. of powdered potassium oxalate to prevent clotting. At the end of 72 minutes after the bladder was first emptied the specimen of urine is collected, carefully measured and used for analysis. A 72 minute period is chosen since it is $\frac{1}{20}$ of 24 hours, and the calculation for 24 hours is made somewhat easier. It should be remembered that the estimated rate of excretion for 24 hours need bear no relation to the amount actually excreted in 24 hours. The rate is actually determined for the shorter period and calculated for 24 hours as a standard period on which to base all results.

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[183] McLean, Jour. Exp. Med., 1915, xxii, p. 212.
[184] Ibid.

Very accurate analyses are necessary if one desires to determine a quantitative relationship such as is here described. The urea content of the whole blood (the urea content of whole blood is slightly lower than that of plasma; whole blood is always used) and of the urine are determined by the urease method. These urea determinations are always corrected by determinations of the preformed ammonia, the amount of which is subtracted.

After the portion of whole blood for the urea determination has been removed the remainder is at once (within 1 hour) centrifugalized at high speed to throw down all corpuscles and the plasma is pipeted off (serum of clotted blood is never used). The total chlorides of both plasma and urine are then determined and calculated as sodium chloride.

The values obtained from the urea and chloride determinations are substituted in the proper formulas as described.

Water is administered before the period in order to prevent apparent retention due to dehydration of the organism. If a fair amount of urine is thus obtained the results in normal individuals will not simulate those of subjects actually retaining urea. Apparently sodium chloride is less dependent on water intake than urea. Diet, especially as regards chloride and nitrogen intake, is unimportant from the standpoint of the observations as the formulas are independent of the intake. It is therefore unnecessary to put an individual on a standard weighed diet in order to obtain comparable observations.

The substitution in the formulas of values found by analysis and the calculation of the formulas is in itself a considerable task if the ordinary arithmetical processes are used. Logarithms are of advantage, but they are laborious. To simplify the process of calculation a slide-rule has been adapted to the formulas. By the use of this device it is not even necessary to remember the formulas; the whole calculation becomes a matter of only a few seconds and is purely mechanical.[185]

The normal concentration of urea in the blood varies in the same or different normal individuals from about 0.200 to 0.500 gms. per liter. The rate of excretion is determined by this concentration and by the rate of water output. It is somewhat less for concentrations below 0.300. The usual range of concentration of chlorides in normal human plasma is from 5.62 to 6.25 gms. of sodium chloride per liter or higher, according to the amount ingested. The rate of excretion depends on the excess over a threshold of about 5.62 gms. per liter. A concentration below 5.62 gms. per liter has not been observed in a normal individual.

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[185] See McLean, ibid.

Renal Permeability to Foreign Substances.—THE METHYLENE BLUE TEST of Achard and Castaigne was supposed to test the "epithelial filtration" ability of the kidney. One-tenth of a gram of this dye in a capsule is given by mouth or 0.05 gm. intramuscularly (1 c.c. of a 1 : 20 solution).

The elimination of methylene blue, first as a colorless chromogen, begins in from 15 to 30 minutes after a subcutaneous injection. In from 3 to 5 minutes later the elimination of the greenish-blue pigment begins. For it to appear only after an hour is pathological. Normally the excretion of this dye reaches its maximum in from 3 to 4 hours and continues from 35 to 50 hours (48 to 60). About one-half is eliminated in the first 24 hours. From the first the test has been severely criticised. In some cases the dye is entirely destroyed in the body and under normal conditions only about 50% of the amount injected is excreted through the kidneys.

INDIGO-CARMINE TEST (VOELCKER AND JOSEPH).—Twenty cubic centimeters of an 0.4% solution of indigo-carmine is injected into the gluteal muscles. The color of the urine of a normal person will change to a greenish-blue in from 10 to 15 minutes after the injection and return to normal in about 12 hours. (That is, its elimination is more rapid than that of methylene blue.) This test is not satisfactory since only about 25% of this dye injected is excreted through the urine and the rest cannot be accounted for.[186]

ROSANILINE (LEPINE).—One cubic centimeter of a 1% solution of rosaniline is injected into the gluteal region. The excretion of the dye begins in about 30 minutes, reaches its maximum in 2 or 3 hours, and continues from 20 to 24 hours. Since from 65 to 95% of this dye is excreted in the urine this test theoretically has much in its favor.

SALICYLIC ACID TEST.—This test was adapted to clinical use by Widal and Ravant as a measure of renal permeability. The excretion of this acid is supposed to be through the glomeruli and to be governed by physical laws alone. One cubic centimeter of a 30% solution of sodium salicylate is injected (with a little cocaine to reduce the pain) intramuscularly. The urine is examined at the end of ½ hour, then hourly, by the addition of a 10% Fe_2Cl_6 solution for its appearance and then the amount eliminated is determined colorimetrically.

Normally the violet color will appear at the end of half an hour. It may even in 15 minutes. It reaches a maximum in from 1 to 3 hours and disappears in from 8 to 12 hours. The amount excreted (*i.e.*, the per cent. of the total) in 5 hours is taken as standard. In the various forms of nephritis the excretion may begin within the first half hour and reach a maximum at the same time in all, but in some cases of interstitial nephritis the output is continued over a longer time. Striking exceptions, however, are reported. This has certain advantages over the similar potassium iodide test since it is simpler and more rapid. Zeigan[187] recommends the quantitative estimation of the salicylic acid output and Singer [188] that of the KI.

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[186] This test is, however, preferred to all others by Thomas and Birdsall, Jour. Amer. Med. Assn., 1917, lxix, p. 1747.

THE PHENOLSULPHONEPHTHALEIN TEST.—Rowntree and Geraghty[189] proposed a test for the functional activity of the kidneys which has proven the best of all. The solution of phenolsulphonephthalein is made up as follows:

Six-tenths of 1 gm. of phenolsulphonephthalein and 0.84 c.c. of $2N$ NaOH solution are mixed with a 0.75% NaCl solution so that 1 c.c. of the solution will contain 6 mgms. of the dye. The result is the mono sodium, or acid, salt which is red in color and slightly irritating when injected. Two or 3 more drops of the $2N$ hydroxide therefore are added which will change the color to a beautiful Bordeaux red.

Twenty minutes to half an hour before the test the patient drinks from 300 to 400 c.c. of water in order to insure a free urinary secretion, otherwise a delayed appearance of the dye may be due to lack of secretion.

By means of a catheter the bladder is completely emptied. Then 1 c.c. of the above described solution is injected intravenously in the upper arm by means of an accurately graduated syringe or injected intramuscularly. The urine is allowed to drain into a test-tube in which has been placed a drop of 25% NaOH solution and the time of the appearance of the first faint pinkish tinge is noted. If the patient has no urinary obstruction the catheter is withdrawn on the appearance of the drug in the urine and the patient is instructed to void at the end of 1 hour and again at the end of the second hour. A rough estimate of the time of appearance of the dye can be made without the use of the catheter if the patient will void at frequent intervals. In cases of prostatic trouble, however, it is wise to leave the catheter closed by a cork stopper in place until the end of the 2 hours. The bladder is thoroughly drained at the end of the first hour and again at the end of the second.

Each sample of urine is measured and its specific gravity recorded. Sufficient NaOH (25%) is now added to elicit a maximum brilliant purple-red color. The urine is now poured into a measuring flask and enough distilled water added to make its volume up exactly to 1 liter. It is then thoroughly mixed, and a small filtered portion used to compare in the Duboscq colorimeter with a standard solution, or to read against a color scale in the Autenrieth-Königsberger hemoglobinometer (sometimes called the "Hellige" after the maker). This instrument as modified by Rowntree and Geraghty has been found easier to use and as satisfactory as the colorimeter.

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[187] Centralbl. f. uni. Med., 1903.
[188] Zeits. f. klin. Med., 1903, xlviii, p. 157.
[189] Jour. of Pharm. and Exp. Therap., July, 1910, vol. i, No. 6.

The standard solution used for comparison is made up by adding 3 mgms. of phenolsulphonephthalein (or ½ c.c. of the solution injected) to 1 liter of water which has been made alkaline by the addition of 1 or 2 drops of 25% NaOH solution. This beautiful purplish-red solution will retain its intensity of color for weeks.

One cup of the colorimeter (right) is half filled with this standard solution and the plunger lowered so that the indicator reads 10. Some of the diluted urine (depending on the intensity of the color) is placed in the other cup, and the plunger manipulated until the 2 halves of the fields have an identical intensity of color. The indicator of the left plunger is now accurately read, and the amount of dye estimated. If, for example, the urine side reads 20 and the standard 10, then the diluted urine must contain only ½ as much dye as the standard solution. To estimate the percentage of dye excreted in the urine one multiplies the reading of the standard by 100 and divides by the reading for the solution containing the urine, *e.g.*, $\frac{10 \times 100}{20} = 50$ which indicated that there is 50% as much dye in the urine as in the standard solution used for comparison. This compares the amount of dye in the diluted urine with that in the standard used for comparison, but to estimate what percentage of the drug administered is excreted one must compare the amount excreted with 6 mgms. rather than 3 mgms. The result in the example given above is 50% of the 3 mgms. or 25% of the 6 mgms. (the amount injected) so that the excretion is 25% of the amount administered. It is possible by this method to detect a difference of 0.04 mgm. in the output of phenolsulphonephthalein.

The standard for comparison described above was chosen arbitrarily because of the beautiful pink color which such solution presents when the indicator stands at 10. The doses injected have varied from 3 to 60 mgms. but 6 mgms. was selected as most satisfactory in the majority of cases.

The objection raised against the quantitative estimation of most dyes by colorimetric methods is that the normal coloring matter in the urine will interfere with an accurate estimation. In the case of phenolsulphonephthalein this difficulty is slight since the color of this dye is so very brilliant. Even from 200 to 250 c.c. of urine may be added to the solution before it is diluted with distilled water to 1 liter without changing the reading. In the case of patients an excretion of more than 200 c.c. of urine an hour would indicate a polyuria and such urine would have a lower specific gravity and a paler color than normal.

If the color of the urine is such that an error might arise from this source one can correct it by adding to the standard solution the same amount of a similar urine as is obtained from the patient. While in this way very accurate quantitative estimations can be made yet in the vast majority of cases such correction is unnecessary since it is not often that a patient voids more than 250 c.c. in an hour. In the majority of cases the technic of the test is simplicity itself.

In normal cases the dye appears in the urine in from 5 to 11 minutes, from 50 to 60% is excreted in the first and from 20 to 25% in the second hour; that is, from 60 to 80% is excreted in the first 2 hours.

The excretion of the drug bears little if any relation to the excretion of water. A high output of dye has accompanied a small amount of urine and the quantity of dye excreted may be small when the amount of urine is great. It is immaterial, so far as the excretion of the dye is concerned, whether the urinary output during these 2 hours is 50, 200, 400 or 500 c.c.

In acute nephritis the functional ability of the kidneys as determined by this test may fluctuate in 24 to 48 hours from one extreme to the opposite.

In many cases of parenchymatous nephritis there is a marked decrease in the amount of dye excreted and this reduction would seem to run parallel to the amount of renal sclerosis present. In 1 case only 10% was excreted in 2 hours. In the early mild cases the function may be but slightly disturbed, while there is 1 group of cases where there is definite hyperpermeability so that 80% and over may be eliminated in 2 hours.[190].

In chronic interstitial nephritis the output is low in practically every case and the decrease is proportionate to the degree of severity of the disease as estimated clinically. In several cases only a trace of the drug—less than 1%—was eliminated in the course of 2 hours. These patients nearly all die within 2 months, many, but not all, with uremic symptoms.

In nephritis, contrary to the normal, the excretion of the dye during the second hour is usually greater than that of the first.

In cases with obstruction in the lower urinary tract, *e.g.*, hypertrophy of the prostate, this test is particularly valuable. These patients frequently have also nephritis, pyelonephritis, pyonephrosis, pressure atrophy, etc. The amount of their urine, its content of urea and of total solids may be practically normal and yet the patient be on the verge of a renal failure which will be precipitated by any operative interference. In these cases the phthalein test will differentiate those with severe from those with slight renal damage. This test has demonstrated the greatest impairment of function in cases with large residual urine and who have not been leading a catheter life. Operations on these men have in the past often proved fatal and yet now may be safely performed after an adequate regime has resulted in a decided improvement of the kidney function, as indicated by this test. When the time of appearance of the dye is delayed beyond 25 minutes and the output of drug for the first hour is below 20%, operation is postponed, regardless of the patient's clinical condition. If under routine reatment the output remains low but constant the renal function is probably in a stable condition and the operation may be cautiously performed. In such a case the operation would be postponed even though 1 test showed a large output of the dye, in order to determine whether or not this condition is stable; for it has long been recognized that the function of the kidney is extremely variable following an operation for the relief of retention. If the further tests indicated a decreasing function, operations should not be performed unless unavoidable, since in our series death from acute suppression sometimes follows operation.

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[190] Baetjer, Arch. of Int. Med., June 15, 1913, vol. xi, p. 593.

Again, operation should not be attempted when but a trace of dye is excreted, since grave renal changes certainly exist. Two patients whose output of dye during 2 hours was but a trace died of uremia within a short period, though at the time of the first test no evidence of uremia was clinically present. In neither case was any operation performed.

In no case in which this test indicated, prior to operation, an efficient or stable renal function has any evidence of renal insufficiency become apparent subsequent to operation.

This test is particularly valuable in determining the functional value of the individual kidneys. The separated urines are obtained by ureteral catheters.

In normal persons the time of the appearance of the drug from the 2 sides is almost the same (from 5 to 10 minutes). The occasional delay of 1 side of 2 or 3 minutes has been noted. In 1 case it appeared in 6 minutes on the left side, and in 25 minutes on the right. In this case, however, there was an anuria on the right side, probably reflex, but the collection of urine for 1 hour showed equal secretions of drug from the 2 sides. In only two normal cases has a distinct difference in the amount of drug excreted been observed.

Seventeen cases of unilateral or bilateral renal infection were studied by Rowntree and Geraghty. Many of these cases came to operation, thus allowing opportunity to estimate the true value of the test.

Unilateral Cases.—When only one kidney is diseased the appearance of the drug on the diseased side is delayed and the amount excreted is relatively and absolutely decreased. The actual time is of comparatively little value since it is the quantity excreted during a period of at least 1 hour which is important. It is possible by extending the observations over a period of 2 hours with the catheters in place to demonstrate with some degree of accuracy the functional ability of each kidney.

In the majority of cases of unilateral disease although the most of the work is done by one yet the combined output is almost equal to that of 2 normal kidneys. Following nephrectomy the 1 kidney will eliminate that amount of drug which would normally be excreted by 2 healthy kidneys and a little more than the combined output of the 2 kidneys prior to operation.

While for practically every functional test great claims are made at first, which later must be greatly discounted, this test alone has thus far stood searching criticism by many workers and is growing in popularity year by year. It is of more value in prognosis than any other single test. While it should not be used to the exclusion of all others, yet in most cases its results indicate with fair accuracy the functional efficiency of the kidney. This test may never do any positive harm and yet that it is not entirely innocuous is evident by the slight increase in pulse rate and the slight fever which follows often and which may last for from 1 to 3 days.

Schlayer's Lactose Test.—Two and one-half grams of lactose dissolved in 25 c.c. of freshly distilled water, in small Erlenmeyer flasks stoppered with cotton, are pasteurized for 4 hours for 4 successive days at 75° to 80° C. Twenty cubic centimeters of the contents of 1 flask (containing therefore slightly over 2 gms. of lactose), the preparation of which has just been completed, is injected intravenously. Only very slight constitutional symptoms follow the injection (a slight headache, malaise, rarely a chill and fever for a few hours). The normal time for the excretion of this amount of lactose is from 4 to 6 hours. The urine should be collected 4 hours after the injection and then for each succeeding 2 hours till 12 hours have passed. The presence of lactose in the urine is determined by Nylander's solution (using for each test the same amount of urine and of reagent and boiling each the same length of time) and its amount estimated by the polariscope.

The mechanism of the excretion of lactose seems to differ essentially from that of phthalein.

Phlorizin Test.—This test of the "secretory ability" of the renal epithelium, rather than of its "permeability" (in the latter function osmosis is supposed to play the important part, in the former, none), was proposed by Achard to replace the hippuric acid test (the ability of the kidney to transform benzoic to hippuric acid—this was a theoretically good test of renal functional ability but clinically useless since the quantitative determination of hippuric acid is so inexact). The phlorizin test is based on the generally accepted opinion that phlorizin diabetes is due to a specific excretory activity of the renal epithelium and that a diminished or absent glycosuria would mean disease of these cells. The bladder is emptied and 1 c.c. of a fresh 1 : 200 solution of phlorizin (hence 0.005 gm.) is injected subcutaneously (a small dose is chosen which will produce glycosuria in only normal kidneys). Sugar is tested for at 15 minute intervals. In normal persons it will appear in from ½ to 1 hour and continue for from 2 to 4 hours. From 0.5 to 2.5 gms. of glucose will be eliminated. In nephritis as a rule less than 0.5 gm. of sugar is eliminated and in some cases none. This test is now considered unreliable. Some normal persons at times do not react at all and the variations in other normal persons may be greater than those seen in cases of renal disease. The test does not permit one to separate the various forms of nephritis, since the "hypoglycosuria," and "aglycosuria" occur with about equal frequency in all forms. Yet it is a test of renal activity and therefore quite different from the other.[191]

The Value of These Tests.—In the medical wards these tests certainly add to the clinical pictures which patients present. By means of them we can follow the results of treatment and in masked renal cases we have no better means of arriving at a prognosis. In preparing patients for operation this test has great value. It is our rule not to transfer any patient for operation with an output of the dye under 50% if the operation can be delayed. The surgeon finds this test of great value, when, *e.g.*, the question is the removal of a diseased kidney. In such a case the first question is, Is there another kidney? The second is, Can this second kidney do the work of both?

Surgeons often get results with this test which are more definitely positive than those obtained in the medical wards. One reason for this may be that surgeons deal more with conditions which destroy all renal function (abscess, cancer, etc.), while among the medical cases there are so many in which many of the functions which can be tested are normal, but that unknown but all-important one, failure of which means uremia. Kümmel [192] stated that in a long experience (in over 500 cases) he had never been deceived by cryoscopy of the blood, while Casper and Richer [193] consider the cryoscopy of the separated urines, together with the phlorizin test (determination of the amount of sugar eliminated by each kidney)—neither test alone but the agreement of both—of actually greater value than the microscopic or gross examination of the renal tissue. We quote these authors merely to show that a skillful man may get great assistance from any test, even a poor one, if only he is thoroughly acquainted with it.

Disturbed Renal Function in Conditions Without Renal Disease.—In certain pathological conditions without definite renal disease the renal functions as measured by dietary tests are similar to those found in patients with advanced chronic nephritis. Christian [194] found this true in cases of pernicious anemia and ascribes it to the anemia, either a nutritional or toxic disturbance of the renal cellular activity. Mosenthal found it true also of cases of prostatic hypertrophy, pyelonephritis and polycystic kidney.

DISEASES OF THE KIDNEYS

At this point it is desirable to define more accurately the elements which enter into our ideas of nephritis. As our nomenclature testifies, it has been the opinion of pathologists that in addition to acute renal inflammations there is also possible an interstitial nephritis, meaning by this a primary proliferation of the fixed tissue-cells of the renal stroma. As clinicians responsible for the lives of our patients we would maintain that in nephritis there are but two processes to be considered; an active destructive process to be fought and a healing process to be favored. We would maintain that in the great majority of cases the kidney suffers only in the discharge of its duty and seldom is it the primary seat of a disease. There is no " vicious circle " which explains the progress of a nephritis. If a nephritis continues it is because a cause (usually chronic infection) elsewhere in the body is continuing and, this removed, no organ can show more brilliant proof of attempts to return to normal than does the kidney. The acute infections or intoxication which injure the kidney are for the most part fed through the blood stream. These injure or destroy renal epithelium and interstitial tissue and the increase in the fixed tissue elements is a conservating process. There are, therefore, 2 processes in progress in each case of nephritis whatever the type, the acute destructive process and the processes of repair. Sometimes the former is very slight but like a slow smouldering fire it may in years reduce the total amount of renal epithelium to very small volume.

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[191] Hugnat and Revilliod, Arch. gén. de. Méd., 1902, vol. viii, p. 19.

[192] Centralbl. f. Chir., 1903, vol. xxx, II, p. 110; also Arch. f. klin. Chir., Bd. 67.

[193] Functional Diagnosis of Kidney Disease, 1903.

[194] Arch. of Int. Med., 1916, vol. xviii, p. 429.

The albumin, casts and renal, pus and blood-cells in the urine are evidence of this acute process. From the rapidity of elimination of various salts and nitrogenous bodies and from the chemistry of the blood-plasma we may judge of the total efficiency of the kidney. The more normal the kidney prior to an acute toxic injury the more spectacular will the result be. This is why the urine in an acute nephritis or following a foot-ball game may give a startling picture: intense albuminuria, casts of all descriptions, hematuria, etc., and yet the prognosis be very good, while the urine of a man dying in uremia may pass as " almost normal " since it contains " only a trace of albumin " and " an occasional cast."

Albuminuria.—Since the presence of albumin in the urine would seem to be the most delicate test we have of renal irritation, we desired first to get a general idea of the incidence of albuminuria, the conditions in which it most commonly occurs and, if possible, to obtain some clue for further investigation. We [195] therefore abstracted the histories of 3631 hospital medical cases with satisfactory urine reports, taking them in order of admission to the hospital without reference to their diagnosis.

It soon became evident that these patients must first be grouped according to age before any classification according to disease was possible.

The age epochs we chose were: from 1 to 15 years, 16 to 25, 26 to 35, etc. The reason for choosing these figures is that the ages of 15 and 25 are more truly transition points in a person's life than are 10 and 20. The sexes also should be studied separately for certain decades at least. On the whole, however, sex has less influence than one might expect. We next divided the cases into 3 groups—those in which the urine was albumin-free throughout their stay in the hospital, those in which the albumin was present for a time but disappeared while the patient was under treatment and those in which albumin was present at each examination. The patients diagnosed as " neurasthenics " probably form a group of hospital patients as nearly normal as any, for this very diagnosis then meant that careful (for that period, 1890–1904) examination had given no evidence of any definite disease. Of the males with this diagnosis the percentages with albumin-free urine were: 1 to 15 years, 100%; 16 to 25, 87%; 26 to 35, 99%; 36 to 45, 90%; 46 to 55, 84%; 56 to 65, 70%; and from 66 years and over, 66%. The drop at the period of adolescence is interesting (see page 227). Of course no one would claim that these patients were normal. Doubtless now with improved methods of examination a positive diagnosis might be easily made in similar cases. Nevertheless this curve of incidence may be used as a base line in judging of the effects of the known disease.

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[195] Jour. A. M. A., Jan. 6 and 13, 1906.

Of the fevers, typhoid after the twenty-fifth year is accompanied by a transitory albuminuria (febrile) in 30% of the cases and a persistent albuminuria in about 30%. One would expect higher figures than this since the fever is so long-continued and bacilluria so common (about ⅓ of all cases). Yet as a disease of the past history, typhoid fever, strangely enough, seems to have injured the kidneys least, notwithstanding the deleterious influence which it has on the peripheral blood-vessels.

Malaria of the tertian and quartan types has little effect on the kidney but estivo-autumnal much. Pneumonia has the highest percentage of transitory albuminuria of all the fevers we studied (in but about 25% of the cases was the urine albumin-free), but it had almost no permanent effect. Pulmonary tuberculosis and acute articular rheumatism cause but little febrile albuminuria. Of the afebrile diseases, the neurasthenics are the best off and those with arteriosclerosis, the worst. In fact, arteriosclerosis seems the one dominating element among the causes of albuminuria.

In those cases which came to autopsy a comparison was made between the anatomical lesions and the urinary findings during life. Cases with marked cloudy swelling, but no other renal lesion of autopsy, had had, as a rule, an albuminuria, usually slight, for 2 or 3 weeks before death. In a few cases the urine was albumin-free even shortly before death. Casts had accompanied the albumin, usually hyaline, but also waxy, epithelial and blood-casts.

Fatty kidneys (no other microscopical changes) had developed in various diseases. Those with fatty infiltration were found in diabetes mellitus, pregnancy, etc.; those with fatty degeneration in cases with various poisons. The amount of urine had been normal in most of these cases although in some severe ones it was decreased in amount; albumin from a trace to a a large amount had been present in every case, but in none for over 2 weeks while casts had been present in a relatively large number of cases: hyaline, granular, fatty, and epithelial. The red corpuscles were few or many in number.

The urine of patients whose kidneys at autopsy showed only chronic passive congestion was at first scanty in amount, dark in color and very acid. Its specific gravity varied between 1.025 and 1.030. The urate sediment was often abundant. Urobilin and uroerythrin were increased and sometimes bilirubin was present. Sooner or later albumin appeared in traces, later in larger amounts, *i.e.*, 0.1% while in 1 case it was 0.6%. Casts were present, chiefly hyalines, rarely the granular, yet on some days the hyaline, granular, waxy, epithelial and fatty casts were present in large numbers. A very few leucocytes also were found and still fewer red cells. The points of importance in the urine of this condition are: the small amount of albumin, the large urate sediment, the absence of renal epithelium and the scarcity of granular casts and leucocytes. A diagnosis of nephritis had been made clinically in over half of these cases.

Acute Nephritis.—The pathologist studying the kidneys describes 10 or more forms of acute nephritis but the clinician finds classification very difficult. Senator separated tubular or acute parenchymatous from an acute diffuse nephritis, not as 2 distinct diseases, but as the extremes of a series of cases which includes every transitional form. In cases of the acute parenchymatous nephritis the tubules especially would seem to be involved; the glomeruli little, or not at all. The clinical symptoms, if any, are slight. The urine is diminished in amount, has a rather high specific gravity, contains but a trace of albumin and few or no casts. A heavy sediment often settles which consists chiefly of renal epithelial cells (hence the name "nephritis desquamativa"). These may occur singly or in casts. In the middle of the series are the cases with hyaline casts, sometimes few, sometimes many, crystals of uric acid and calcium oxalate, red blood-corpuscles, hemoglobin in granular casts or masses and a few leucocytes in the sediment. In some the small amount of albumin presents a remarkable contrast to the large amount of sediment and may be chiefly Mörner's body. At the other extreme of this series are those of acute diffuse nephritis, a good illustration of which is that following scarlet fever. In this clinical symptoms are much more severe. The urine is diminished in amount; there may, indeed, be anuria for the first 24 hours. Other cases void from 50 to 100 c.c. for the first day or so and later from 200 to 500 c.c. Toward death the amount may be diminished or increased. The specific gravity is normal as a rule, from 1.015 to 1.017, but in some cases is high, from 1.023 to 1.025 (when the amount of urine is from 300 to 600 c.c.) while in the cases with very scanty output (under 500 c.c. in 24 hours) it may reach 1.030. The urine is usually of a dark color and cloudy, but in very mild cases it may appear normal. Blood is practically always present, in traces or enough to impart to the urine a slight smoky tinge. Some urines are reddish brown, brownish or even of a chocolate color depending on the amount of blood present and on the proportion of the hemoglobin which has been transformed to methemoglobin. The albuminuria is usually intense and yet in some cases, even fatal ones, mere traces may be present and these but for a few days, alternating even till death with periods during which the urine is albumin-free. Serum albumin and serum globulin both are present, and if many cells are in the sediment a certain amount of true nucleo-albumin and of albumose. Albumose in some cases is the only proteid found. Why, is not clear. This may explain, however, cases described as "albumin-free," since the examiner may have used only the heat and acid test, which would not precipitate albumose. As a rule not above 1% of albumin is present and of this considerable is globulin. Red blood-cells may always be found in the sediment, also mononuclear cells, a few polynuclear leucocytes and epithelial cells from the urinary tubules which may be single or in masses and which usually are very fatty. Among the crystals met with are uric acid and calcium oxalate. Hemoglobin may be present either in amorphous granules or in casts. The leucocytes were very abundant in 1 case of acute nephritis with multiple abscesses. The number of casts varies much from day to day. Sometimes they are present in enormous numbers and in all forms. The epithelial, hyaline and coarsely granular casts predominate, but blood and leucocyte casts may also be present. As a rule the number of casts runs roughly parallel to the amount of albumin. In 1 case of acute hemorrhagic nephritis with areas of complete necrosis the amount of albumin just before death was slight but the number of casts, including leucocyte and granulars, was large. In 1 case of general septicemia traces of albumin were present in the urine on some days, none on others; and yet this urine often contained blood cells and hyaline and leucocyte casts.

During the course of a case of nephritis the urine shows every symptom of renal insufficiency. The nitrogen output, apart from variations due to the diet, is diminished; the output of chlorides and phosphates is low hence the molecular concentration of the urine is less than normal. The uric acid output is about normal while that of the xanthin bases is said to be increased. The ability of the kidney to form hippuric acid is diminished and the glycosuria after phlorizin injection is either slight or absent. The phenolsulphonephthalein test gives varying results but in general gives a fair idea of the severity of the disease at the time the test was made but little as to prognosis. The renal test day may be quite misleading. The blood-urea estimations give the most consistently valuable means of determining the degree of the progress in any given case.[196] In mild cases, and in severe ones as they improve, the urine is nearly normal. It is said that in acute renal infection the albumin disappears last but we believe the casts are more often found later than is albumin.

Nephritis Hemoglobinuria.—In acute nephritis the urine may contain considerable hemoglobin and few or no red blood-cells. In certain cases a hemoglobinuria would seem to be the cause of the nephritis, in others it is a symptom. The former may be true of cases of hemolysis due to poisons, burns, etc., while severe cases of the infectious diseases, especially typhoid fever, scarlet fever, malaria, Winckel's disease of the new-born, etc., may cause a nephritis with hemoglobinuria or nephritis and hemoglobinuria. Nephritis hemoglobinuria differs from pure hemoglobinuria in that in the former the amount of albumin is greater and the sediment richer in casts, renal epithelial cells, leucocytes and uric acid crystals.

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[196] Atchlay, Arch. of Int. Med., Sept., 1918, xxii, p. 370.

Acute Nephritis of Cholera.—With Asiatic cholera is said to develop a peculiar type of pure parenchymatous nephritis of the tubular variety. The urine is diminished in amount, in fact there may be anuria for from 5 to 7 days. It is dark and cloudy, but rarely bloody, very rich in salts and may deposit a large urate sediment. Albumin is present in relatively larger amounts than in the other forms of parenchymatous nephritis. Hyaline and granular casts, renal epithelium, red blood-cells, leucocytes, uric acid and calcium oxalate crystals are found in the sediment. The urine is characterized also by its richness in the ethereal sulphates, the frequent presence of acetic acid and the increased amount of ammonia. The acidosis in these cases may be severe. One patient recovered after an anuria of 15 days. The condition of the urine improves much during the stage of reaction.

Nephritis syphilitica acuta precox is sometimes marked by an intense albuminuria. In 1 case (Hoffman and Salkowski) the urine contained 8.5% of albumin and coagulated to a solid mass when boiled. This urine had very little sediment, only a few casts, leucocytes and blood-cells.

Subacute Nephritis; Chronic Parenchymatous Nephritis; Chronic Diffuse Non-indurative Nephritis; Large White Kidney.—This form of subacute nephritis, which may follow an acute nephritis or develop insidiously, is characterized clinically by its subacute course (it is usually fatal within 2 years) by the extreme anasarca and effusions into all the serous sacs, its incidence especially in young persons who work hard amid exposed, unhygienic surroundings and its frequent association with certain constitutional diseases, as tuberculosis, lues and malaria and with chronic alcoholism.

The amount of urine during the acute stages of this disease is diminished to about 250 to 500 c.c. in 24 hours, the diminution varying as the edema. This is especially marked just before death. As the case improves, however, the amount increases, and if the patient be encouraged to drink fluids he may void from 5 to 6 liters of a very dilute urine each day. The amount is increased also when the edema or the effusions begin to absorb. Its specific gravity, which varies inversely as the amount, is, as a rule, almost normal or slightly increased, in some cases reaching even 1.040. Its reaction is faintly acid, but in some cases it is alkaline when voided and in all cases it quickly becomes so on standing. This makes the search for casts difficult. Its color varies from a pale greenish-yellow to a red or a reddish brown. It is cloudy as a rule from the large amount of sediment present and foams easily on shaking because of the considerable amount of albumin it contains. Profuse painless hematuria may be a feature of this form of nephritis and unfortunately so far as diagnosis is concerned may be unilateral, first from 1 side then from the other.[197]

In this form of nephritis the albumin in the urine is abundant both relatively and absolutely. It varies roughly as the specific gravity and seems to bear no relation to the amount of edema present. It seldom reaches 1% and for months may vary from 0.4 to 0.8%. In certain cases, however, it reaches 2% and Bartels reported one in which it varied from 4 to 6%. The albumin quotient varies much. Nucleo-albumin is present in small amounts, also albumose. Some of these cases develop into the chronic indurative type in which cases the amount of albumin diminishes progressively.

The output of urea is somewhat diminished even when there is much dropsy. That of uric acid varies, but remains within normal limits. The ammonia is normal. There is a certain retention of chlorine and of phosphoric acid.

The urine sediments in this condition resemble those of acute nephritis, but one finds more coarsely granular, fatty and waxy casts. Red blood-cells are always to be found and during the acute exacerbations many. There is little difference between the urine of the white and of the mottled kidneys except, perhaps, that in the latter are found more red blood-corpuscles, leucocytes and fatty cells.

These kidneys show some functional insufficiency and yet in even the severe cases they do their work fairly well. The explanation for this is that the disease attacks locally successive parts of the kidney and that while 1 part is inflamed other parts can carry on the renal work.

One would expect that an involvement predominantly glomerular would produce an intense albuminuria while one predominantly tubular a marked cylindruria. While in general this may be true clinically it is of little importance since both tissues are always involved.

Except in the case of very young persons with a past history of fine health the diagnosis of subacute parenchymatous nephritis has difficulties since patients with an acute exacerbation of a latent chronic nephritis may present similar clinical features and may void a very similar urine and yet at autopsy small contracted kidneys be found.

Chronic Indurative Nephritis.—A clinical subdivision of the group of cases which at autopsy have small contracted kidneys is exceedingly difficult. In fact even at autopsy the size and color of the kidney, and not the histological pictures, are the only safe basis of classification. Also, the anatomical conditions of the kidney may be of little help in interpreting the preceding clinical course of the case since the same end result may be reached by different pathological processes (Christian).

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[197] Kretschmer, Ill. Med. Jour., Aug., 1912.

Some have considered the so-called " senile atrophy," as almost physiological in elderly persons, claiming that the kidney grows slightly sclerotic with age. Nascher [198] for example says that " senile contracted kidney with slightly diminished output of urine of rather high specific gravity and a trace of albumin without casts is a physiological condition. It requires no treatment." Others however believe that these changes are common not because these patients are merely beyond middle life but because they have for more years harbored infected noses, bad tonsils, and infected mouths (pyorrhea alveolaris) than in the case of younger persons. If the process in elderly persons were merely an atrophy there should be few qualitative urinary changes. But as a result of hard work (possibly), of chronic infections, especially those of the nose and mouth and colon, of various diseases, especially gout and lues and of certain poisons, as lead and alcohol, insidious slow inflammatory and degeneration processes develop. The result of these is inflammation and degeneration of the epithelial elements and this may be general or focal, subcortical or periglomerular and with a subsequent compensatory new growth of connective tissue. The kidney becomes hard and firm, it shrinks in size and finally is but a remnant of an organ. Such kidneys show that a nephritis can heal, for we found at autopsy markedly contracted kidneys which could not possibly have been suspected from the urine voided before death. It is evident from the history that some of these cases are the result of a preceding acute or subacute nephritis. Other cases die with all the symptoms of an acute nephritis and we to our surprise find at autopsy evidence of a marked nephritis of years duration of which the final illness was an acute exacerbation.

Even the pathologist cannot find any definite basis of classification of these contracted kidneys except their weight, the thickness of the cortex and their color, and so have divided them into the " red " and " white " kidneys. In the red kidneys the arterial changes are a prominent feature. They are firm and beefy, the disappearance of the epithelial elements is extensive and the amount of fibrous tissue considerable. Yet these kidneys are seldom as small as are the white. The latter kidneys are very small, are pale yellow in color and conspicuously fatty. Little cysts are numerous in the cortex. The student should be reminded that a considerable sclerosis of the smaller blood-vessels of the kidney, extensive enough to produce malnutrition of the renal epithelium, will lead to atrophy and sclerosis of the kidney; and that while this process is not nephritis yet it explains many of the lesions of nephritis. In each kidney there are 2 reasons for the connective tissue proliferation: the death of epithelium as the result of direct toxic action and the starvation of epithelium as the result of vascular disease. The latter process may be in part local and 1 kidney therefore suffer much more than the other. Christian[199] after years of study finds himself justified in making the diagnosis "chronic interstitial nephritis" and "chronic glomerular nephritis" less and less often and now more often makes the diagnosis "chronic nephritis with or without hypertension."

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[198] N. Y. Med. Jour., June 24, 1916.

Chronic Interstitial Nephritis.—In chronic interstitial nephritis the renal tissues have suffered from a slow chronic infection which has gradually reduced the amount of secreting tissue until but very little may be left. These are the cases in which the acute element is slight but since it is in operation for years it destroys an immense amount of kidney tissue. These cases are marked by their very insidious onset. The only symptom for years may be a slight albuminuria with perhaps a few casts and these may be absent for long periods of time. The amount of urine is increased slightly at first, but later in a well-developed case from 2 to 3 liters and sometimes even 12 liters are voided daily. On the other hand it may at times sink to normal or even under. The urine is pale, clear, definitely acid and has a specific gravity constantly between 1.010 and 1.005 irrespective of how much water the patient drinks. This fixation of specific gravity shown clinically by the low specific gravity of the morning urine is always significant of this condition. The molecular concentration is diminished. The amount of albumin seldom rises above 0.05%, and usually is much less. It is often absent in the morning voiding and may indeed be present only after a day of unusual exercise or an especially hearty meal or some unusual excitement.

Hyaline casts can usually be found in the centrifugalized sediment. Red blood-cells are very common in the sediment. Sometimes there is definite hematuria. There is often sufficient desquamation of the epithelium cells of the urinary tract to produce a cloudy urine resembling that of cystitis.

In the cases of arteriosclerotic kidneys the albuminuria appears late and is often intermittent. The most of the cases of so-called "contracted kidneys with albumin-free urine" belong here, and in these cases albumin is found even more constantly and in larger amount than in cases of the preceding group. During periods of improvement the casts often disappear first leaving a pure albuminuria while in the group to which the small white kidneys belong the albumin often disappears first.

The output of nitrogen is practically always normal but the percentage of the various nitrogenous bodies may vary somewhat. In uremia, *e.g.*, the ammonia may rise at the expense of the urea. Uric acid is low and the xanthin bases are increased. The tests for functional renal efficiency sometimes indicate a pathological insufficiency but more often do not. The sediment is scanty and difficult to find. After a long search but 1 or 2 casts may be found in a centrifugalized sediment. These usually are hyalines although sometimes they are finely granular casts. Sometimes one finds a few renal epithelial cells and a few leucocytes, and more often than one would think a few red cells, especially after exertion. Uric acid and calcium oxalate crystals are common in the sediment.

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[199] Cleveland Med. Jour., April, 1917.

During acute exacerbations of a chronic nephritis the urine may closely resemble that of more acute nephropathies.

For the diagnosis of chronic nephritis one should examine the morning and the evening urines separately and also that voided after severe exercise. The urine of these patients may resemble so closely that of other conditions (*e.g.*, the convalescence of acute or subacute nephritis, waxy kidney, and the cyclic, or " physiological " albuminurias) that the clinical history and the physical examination of the patient are necessary for diagnosis. The tests for renal function are of great value in these cases in determining diagnosis, prognosis and treatment but must be interpreted in the light of the clinical findings.[200]

AMYLOID DEGENERATION is a condition which may be superimposed upon any form of nephritis, of which it really forms no part. When the kidney is merely waxy, the urine is said to be normal. In the majority of cases the condition accompanies a nephritis and could not be suspected from the urine alone, the examination of which would suggest, when concentrated, chronic passive congestion; when dilute, small contracted kidney. The classical description of the urine of waxy kidney is that it is increased in amount, is pale, clear, faintly acid, of a low specific gravity, 1.005 to 1.012, that it contains abundant albumin with relatively much globulin and very few casts. This picture of Traube, however, is rare. The albumin may occur in traces or fail and the casts may be numerous. The casts are often fatty. Renal epithelium is seldom seen and red blood-corpuscles are extremely rare.

Uremia is the name given a syndrome usually associated with severe renal conditions and considered the highest expression of renal insufficiency, the most marked features of which are cerebral in origin; confusion, mania, gradually developing coma and often convulsions. The name was given by Bright who found in such cases the blood urea much increased and who supposed this the toxic substance involved. In chronic nephritis the body would seem to become tolerant to the renal insufficiency, for uremia is less common in chronic than in acute nephritis. The interesting experimental work of Folin, Karsner, and Denis,[201] emphasizes the importance of glomerular rather than tubular lesions in the production of that nitrogen retention which, it is supposed, explains uremia.

But renal insufficiency alone, even though lethal, does not explain uremia. There can be no more complete renal insufficiency than that which follows the removal by operation of all the kidney tissue a patient has, as when the surgeon excises the diseased kidney of a patient whose other kidney had previously been destroyed, or removes a double kidney, or which follows bilateral calculus or bichloride poisoning. In these cases death may be delayed for from 10 to 14 days, during which time and even until the last hour the patient's mind is quite clear and he shows to the end none of the symptoms usually associated with uremia. Again, we see patients with chronic nephritis who, for even 3 weeks before death, voided a normal amount of urine which was almost normal as judged by the ordinary tests, but who eliminated practically no phenolsulphonephthalein in 2 hours and whose blood-creatinin was well over 10 mgms. per 100 c.c. of blood, and who were clear of mind until the last hour or so of life. This is good evidence that the retention of urinary constituents alone is not enough to explain the coma or convulsions of uremia.

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[200] Christian, The Jour. of Urology, June, 1917, I, No. 3.

[201] Jour of Exp. Med., Dec. 1, 1912, vol. xvi, p. 789.

Foster [202] has clarified our ideas much by dividing the cases of severe renal insufficiency into 3 groups: (1) the retention type, the urinary poisoning of Ascoli, in which practically all the constituents of urine are retained. This type follows the removal of all the functioning renal tissue, mercuric bichloride poisoning, impacted renal calculus, etc. In these cases there are no mental or nervous features, no convulsions and no gastro-intestinal symptoms until the very end. These cases present a pure type of urinary poisoning. A somewhat similar type is seen in cases of small contracted kidney with arterial hypertension, the symptoms beginning with the terminal cardiac decompensation. The picture develops more slowly than in the above cases and ends with asthenia, anorexia, a mild delirium and stupor. (2) The cerebral edema type. The second type is more often seen in cases of large white kidney (subacute parenchymatous nephritis) which renal disease leads to retention of only certain of the urinary constituents, especially of the water and salts. In these cases the cerebrospinal fluid is under increased pressure, there is edema of the retina and at autopsy edema of the brain and meninges. These cases when they develop uremia have vomiting, headache, stupor, amaurosis due to retinal edema and finally coma, but very seldom convulsions.[203] (3) The toxic type or epileptiform uremia. In this, the classical type, there is in addition to the nitrogen retention the presence of some toxin which leads to convulsions. This is the uremia of most authors.

Foster emphasizes the scarcity of pure types of these forms of uremia, the features of more than one usually being present.

One group of 10 of our cases was interesting since it suggested that uremia may sometimes be followed by an apparent improvement in the patients' condition. In 8 of our cases of terminal uremia the albumin increased before death. In 1 case of uremia there was but a trace of albumin on the day before and on the day following the convulsion, but on the day of the convulsion the albuminuria and the cylindruria were intense.

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[202] Jour. of A. M. A., 1916, vol. 67, p. 927.

[203] See Trans. of the Assoc. of American Phys., 1915.

In eclampsia the urinary features are similar to those in epileptiform uremia.

The temporary character and the extreme grade of the albuminuria one meets with in eclampsia are striking. In 1 case in the maternity ward the urine at 10 A.M., March 6, contained 0.653% albumin (gravimetrically determined). The woman was then in the first stage of labor and it was then that convulsions began. The urine between 10 A.M. and 5 P.M. of that day contained 1.23%; between 5 P.M. and 9 P.M., 0.19%; at midnight, 0.075%; at 3 A.M., March 7, 0.025%; and for the rest of that day and later merely a trace.

In another case the total albumin was 0.4678 gm. per 100 c.c., of which the globulin was 0.16 gm. per 100 c.c. (34%). In still another case the urine contained 18 gms. of albumin per liter, a multitude of casts and renal epithelium and yet at autopsy the kidneys presented no evidence of severe trouble.

Unilateral Nephritis.—In the Johns Hopkins Hospital series of cases of nephritis followed to autopsy there was no case of strictly unilateral nephritis, but there were at least 30 cases with a considerable inequality in size of the 2 kidneys and in 3 the difference was marked. In these 3 cases the combined weights of the kidneys were 155, 190 and 205 gms. and the difference in weight between the 2 organs respectively 45, 50 and 65 gms. In a very interesting case at operation Dr. Kelly found unilateral suppurative nephritis. Reisman and Müller [204] have discussed this subject at length. They say that acute unilateral inflammations differ from the commoner types of acute nephritis in that they are interstitial in character, are inflammatory rather than degenerative in nature and have a marked tendency to abscess formation. These cases occur in connection with scarlet fever, erysipelas, osteomyelitis, endocarditis, pyemia, pyelitis, etc.; that is, as part of a general infection and are the result of bacterial invasion of the kidney.

Renal Atrophy.—Renal atrophy may be due to insufficient blood-supply, to cachexia, the anemias and especially to advancing age, the so-called "senile atrophy." One sees microscopically no great increase in connective tissue. The urine is practically normal and without albumin.

Congenital Cystic Kidney.—The urine in the very rare condition of congenital cystic kidney may be normal but more often resembles that of small contracted kidneys. Its amount is increased, its specific gravity low, a trace of albumin may or may not be present while usually the urine contains considerable blood. The contents of these cysts are not at all uniform, not even in those of the same kidney. In some the fluid is clear, watery and almost colorless, in others it is milky or colloidal; some cysts contain urea even in large amounts, also uric acid, while others contain none. In some have been found cholestrol crystals, colloid or proteid-like masses and rosette masses which resemble leucin.

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[204] Arch. of Int. Med., June 15, 1913, vol. ii, p. 601.

Suppurative Nephritis.—The urine of cases of suppurative nephritis contains albumin in varying amounts and but few casts. In the sediment of 1 case there were a great many red blood-cells and leucocytes while in that of another there were very few leucocytes. When the pus-cells are numerous the urine will be alkaline. In a very few cases the urine has contained fragments of renal tissue.

In cases of purulent nephritis the amount of pus in the urine may be disappointingly small since a kidney with an abscess which involves the whole organ may excrete no urine. Sometimes the kidneys are studded with renal abscesses and yet the urine quite free of pus since none of these abscesses communicate directly with the tubules.

Cancer of the Kidney.—Hematuria is often an early, even the first, symptom of cancer of the kidney provided this involves a pyramid. Hematuria was a feature in ½ of the Hopkins cases and the first symptom in ¼ of them. The amount of blood voided may vary from a very slight trace to a fatal hemorrhage, which may be intermittent or of long duration; the blood may be fresh or decomposed, while clots even of large size may be voided. Otherwise the urine of these cases is practically normal.

Tuberculosis of the Kidney.—Cases of general miliary tuberculosis usually have no urinary symptoms and those which may be present are not due to the tuberculosis alone. In tuberculosis of the pyramids with the formation of large caseous masses which may break down leaving a cavity, the so-called " renal phthisis," the urine is similar to that of pyelonephritis except that caseous material may be found in the urine. If the pelvis is not involved there may be no urinary changes. In very early cases of renal tuberculosis polyuria with or without albuminuria is often an interesting feature. Hematuria also may be the first symptom in such cases and was present in 8 of the 17 cases reported by Dr. Walker.[205] This early hematuria is very seldom a marked or serious feature and may last for months. It is present both day and night and bears no relation to the position of the patient, hence differs from that due to calculus. On the other hand it may be so severe as to be a serious feature. Blood-clots often appear in the urine. Pus was present in 15 of the 17 cases, sometimes a little, sometimes large amounts, depending on the position of the cavity. The sediment may contain (in 9 of the 17 cases) tissue detritus in masses about the size of a grain of sand in which are found tubercle bacilli and elastic tissue. Albumin was present in 16 and casts in 6 of this series. One should not be misled by the perfectly normal urine which may be excreted during the days on which no urine comes from the diseased side.

In general it may be said that in all cases of hematuria and pyuria, especially if the urine is acid, tuberculosis of the kidney should be excluded. For diagnosis the tubercle bacilli themselves must be found. And yet since these bacilli can be excreted through a " practically normal " kidney tuberculosis of other organs must also be excluded. If a focus of this disease does not ulcerate into the pelvis of the kidney the entire organ may be destroyed before the condition is suspected.

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[205] Johns Hopkins Hosp. Rep., vol. xii.

Infarction of the Kidney.—An intense albuminuria which begins suddenly, which disappears soon and which is associated with no abnormal sediment strongly suggests renal infarction. One usually finds, however, evidence of a preceding nephritis. The sediment usually contains red blood-cells but a marked hematuria is rare.

In cases of bilateral infarction there may be oliguria and even anuria.

Pyelitis and Pyelonephritis.—Inflammation of the pelvis of the kidney may be due (1) to an infection ascending along the ureter, to a descending renal infection, or to an infection extending by contiguity from neighboring organs; (2) to local causes, as stone, cancer, tuberculosis, parasites (echinococcus, amebæ, etc.), trauma and floating kidney; or (3) to systemic causes, especially to the specific toxins of acute fevers, to medicines, etc. It is usually unilateral.

The symptoms of pyelitis are usually masked by those of the disease of which this is a complication; but even when the possibility of a pyelitis is realized there may be so few local symptoms which indicate it that ureteral catheterization is necessary.

The urinary features in pyelitis will depend on its cause. Sometimes there is anuria (due to the reflex influence of the diseased over the sound kidney) while in chronic cases the amount of urine is sometimes even trebled. The urine contains but little albumin. It is cloudy from the presence of pus, blood and mucus, and faintly acid unless it has undergone ammoniacal decomposition. In the diphtheritic form of pyelitis, the urine will contain fibrin threads and even casts of the pelvis of the kidney.

In the pyelitis of infancy due to Bacillus coli there may be no pus in the urine until a few days after the temperature has begun to rise.[206]

Microscopically the urine contains red blood-cells, mucous fibers, pus and various epithelial cells; uric acid and calcium oxalate crystals, also phosphate crystals if the urine is alkaline when voided; fibrin coagula, tissue constituents and other elements suggesting the cause of the trouble, as tissue fragments, tumor fragments or parasites. All forms of the epithelial cells of the transitional epithelium will be present. It is possible that a preponderance of cylindrical tailed cells may suggest the renal pelvis as the seat of the inflammation (see page 264 and Fig. 51, *a*). We have found many of these cells, often in tile-shaped clusters, in the urine of several cases of pyelitis, but in 1 very acute case of pyelitis with autopsy the urine contained none. There will be no casts or renal epithelial cells in case nephritis also is not present.

A particularly important sign of pyelitis is the variation in amount and quality of the urine. The temporary obstruction of the diseased side will explain the periods with normal urine followed by periods with urine from the affected side.

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[206] Thomson, Quart. Jour. Med., 1910, vol. iii, No. 11.

In the diagnosis of pyelitis the absence of disturbance of micturition is of great importance, also the homogeneous distribution of the pus in the urine and the club-shaped tailed cells in groups with a tile-like arrangement.

In **hydronephrosis, pyonephrosis** and **uronephrosis** the urinary symptoms of importance (apart from the pus) are the variations in the amount of urine, the periods of oliguria alternating with polyuria and the sediment the constituents of which will depend on the health of the cortex.

Renal Calculus.—During an attack of renal colic the urine may be normal in amount or complete anuria may prevail. When, however, the obstruction is relieved, blood, mucus and pus will be voided with the urine.

In addition to the colic, hematuria is a very common symptom of renal calculus (especially of the oxalate stones). Sometimes a clot of blood is passed, sometimes and especially early in the case, the hemorrhage is profuse. Later the symptoms are those of pyelitis.

With ureteral calculi are associated hematuria and oliguria, followed by po yuria. The oliguria was a feature in about 25% and anuria in about 16% of Schenck's cases.[207]

Parasitic Diseases of the Kidney.—In echinococcus disease of the kidneys the only renal symptom may be a mucous catarrh of the pelvis, which later may become a purulent or gangrenous pyelitis. When a large hydatid cyst ruptures into the urinary tract there suddenly is voided a watery (or soapy, milky, or bloody) fluid. While the cyst is discharging the hooklets, scolices, fragments of membrane, etc., may be found in the sediment.

For other parasites, see page 306. Pyelitis and even renal atrophy may be due to Bilharzia infections (page 306).

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[207] Johns Hopkins Hosp. Rep., vol. x, p. 477.

CHAPTER III

THE STOMACH CONTENTS

THE VOMITUS AND GASTRIC CONTENTS

THE various **forms of vomiting** have been grouped as follows: CEREBRAL, in brain and cord disease, as tabes, insular sclerosis, meningitis of brain or cord, cerebral anemia or hyperemia, concussion of the brain, brain tumors, etc.

TOXIC: opium, tobacco, ether, chloroform, alcohol, uremia, cholemia, pregnancy, etc.

PERIODIC, "CYCLIC," OR "RECURRENT" VOMITING.—These cases are characterized by the periodic recurrence of sudden attacks of vomiting, often without apparent cause, which are sometimes accompanied by intermittent hyperchlorhydria. There is evidence that some of these cases which resemble a secretory neurosis, especially those of children, are due to an acidosis, *i.e.*, to an autointoxication.[1]

NEURASTHENIA AND HYSTERIA.—One interesting case of neurasthenia vomited repeatedly from 3 to 4 ounces of bile-stained fluid in from 3 to 4 hours after the stomach had been washed out.

REFLEX: as in peritonitis, strangulation of the bowel, sexual disturbances, chronic obliterative appendicitis, cholelithiasis, renal colic, intestinal worms, eye strain, etc.

LOCAL: due to gastric conditions, whether acute or chronic, and especially those with stasis of the gastric contents.

The Vomitus and General Considerations Concerning the Gastric Contents.—Considerable information, chiefly of a negative character, may sometimes be gained from the inspection of the vomitus. Its microscopical study seldom is valuable since we did not control the food ingested, while its chemical examination is often misleading for we seldom know the previous condition of the stomach, nor the character of this meal, nor can we control the time the food was in the stomach, nor evaluate the effect on digestion of the condition which led to the emesis, nor exclude the mucus and saliva from the mouth.

THE REACTION OF VOMITUS, with the exception of a few cases of achylia and of cancer of the stomach with alkaline gastric contents, is acid to litmus, provided the food had been in the stomach for at least half an hour, unless there had been a marked regurgitation of duodenal contents. This is of importance in excluding diverticula of the esophagus, in which case the vomitus may contain no gastric juice at all. Free hydrochloric acid is seldom present except in nervous cases since the conditions leading to the vomiting usually are those which would depress gastric secretion.

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[1] Edsall and Snow, Am. Jour. of Med. Sci., 1904, vol. xxviii.

The character of the vomitus is important. Abundant, thin, acid vomitus containing food eaten the previous day means dilated stomach due to pyloric obstruction; very watery, thin, acid fluid with finely divided fragments of the food suggests ulcer; thick vomitus containing much mucus and pieces of poorly digested often decomposing meat suggests chronic gastritis and cancer of the stomach; recently eaten, undigested food suggests nervous vomiting. If the vomiting occurs at the height of digestion and during a paroxysm of pain which seems relieved by the vomiting one thinks of ulcer; if during or shortly after eating, of cancer, catarrh, or a neurosis; and if independently of eating (*e.g.*, before breakfast) and contains not only mucus and bile but also food remnants, of gastric ectasis. Cerebral vomiting is often marked by a noticeable absence of effort; morning vomiting is suggestive of pregnancy, or, in the case of men, of alcoholism. In case of cancer or any other stenosis-producing disease at the cardiac orifice the vomiting immediately follows a meal; but if at the pyloric orifice, the vomiting occurs at least 3 hours after the meal and has a volume much greater than that of the meal.

A small amount of blood in the vomitus is of no account since the effort of vomiting easily causes slight trauma of the esophagus or pharynx.

Bile and Pancreatic Fluid.—Traces of bile are often present in the vomitus from a fasting stomach and in that raised with great effort. It has no significance unless it is constantly present and in vomitus expelled without strain sufficient to force bile from the duodenum into the stomach in which cases it might suggest stricture of the duodenum below the ampulla. A green vomitus does not always contain bile,[2] for a " grass-green," " sea-green " or " dark-green " color may be due to the presence of algæ or to chlorophyll-containing protophytes. One reason why vomitus from an empty stomach is more apt to contain bile than is that from a full one is that in the latter case the pylorus is more apt to be in tonic contraction. This may explain the former belief that in peritonitis the vomitus is more often bile-stained than in cerebral troubles, since in the former condition the stomach is often quite empty.

Mucus is usually present in vomitus in large amounts. There are several reasons for this. One is that inflammations of the stomach wall are common causes of vomiting; another, that while the amount of mucus secreted was normal the usual amount has not yet been digested; while a third is that the increased secretion of mucus represents a protective phenomenon, the wall thus shielding itself against chemical trauma, or from pus swallowed from the nose and mouth. This may explain why the vomitus of alcoholics usually contains large amounts of mucus.

The vomiting of large amounts of acid gastric juice, sometimes pure, sometimes mixed with food, is common in cases of hypersecretion, especially in cases of gastroxynsis, a neurosis with periodic attacks of vomiting of acid fluid. The proteid of the food in such vomitus will be well digested, the starch less so.

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[2] Kuhm, Zeitschr. f. inn. Med., 1902, No. 28; 1903, No. 1.

The vomiting of large amounts of fluid containing *food eaten 2 or 3 days previously*, the proteid of which is poorly digested and the starch well digested, suggests malignant stricture of the pylorus while if the proteid is well digested and the carbohydrates poorly, this would suggest benign stricture, due to ulcer, etc.

Fecal vomiting usually indicates a complete obstruction of the ileum or the colon, or paralysis of the intestinal wall due to peritonitis, etc. Such a patient vomits repeatedly, each vomitus a little more fecal than the former. That from the colon is black, foul-smelling and contains vast numbers of bacteria. Yet the absence of fecal vomiting would not exclude a total obstruction high in the jejunum and some hysterical patients have vomited the contents of the colon. For the vomitus to have even a suggestive fecal odor the obstruction must be at least 6 feet from the pylorus.

Rice-water vomitus, seen in Asiatic cholera, is very watery in character and is filled with white flakes of mucous shreds and epithelial cells (see page 422).

From the color and the odor of the vomitus a diagnosis of poisoning or alcoholism may be suspected. That of uremia has an ammoniacal odor.

Some idea of the *motility of the stomach* may be obtained from an inspection of the vomitus although temporary disturbance of gastric motility may be expected in many cases with conditions which would lead to vomiting. If food is vomited 7 hours or more after the last meal, gastric motility is certainly delayed, temporarily at least. At the end of 1 hour bread should have been broken up to a fine, crumbly sediment, which settles to the bottom of the glass. If the vomitus contains large particles of bread, and especially if these are coated with mucus, the secretion of hydrochloric acid is quite surely diminished.

The *chemical analysis of vomitus*, as stated above, is exceedingly unsatisfactory. If free hydrochloric acid is present in vomitus, we may be sure that it is present in this case under more normal conditions; but if absent we can draw no conclusions. In general it may be said that normally both free hydrochloric acid and pepsin are present in the gastric juice 2 hours after a mixed meal.

Tumor fragments have been found in vomitus but this is rare. In the vomitus may be found also round worms, segments of tapeworm, oxyuris, maggots, etc.

Examination of the Fasting Stomach.—While the normal fasting stomach should theoretically be empty yet as a rule one can syphon off through a stomach tube from 1 to 50 c.c. of acid gastric juice. There is some difference of opinion concerning the amount which should be considered the upper limit of normal for the fasting stomach, yet many agree with Boas that 100 c.c. or more of fluid certainly is abnormal and would indicate hypersecretion or motor insufficiency. Which, may be decided by washing the stomach out at night and passing the tube the next morning. If the case were one of motor insufficiency the stomach will be quite empty. Riegel insists that the normal fasting stomach is always empty and that to find even a little fluid is pathological.

The fluid from the fasting stomach is watery with a specific gravity from 1.004 to 1.005. It contains some free hydrochloric acid but not lactic acid and no bacteria. In many cases it is bile-stained, but this is not important unless it is found so on several examinations, in which case duodenal stricture may be suspected. If alkaline from the presence of pancreatic juice, trypsin may be tested for. To find trypsin in an acid or neutral fluid soda must be added at once to prevent the destruction of this ferment. Abnormal amounts of mucus may be present in cases of anacidity, atrophy of the mucous membrane, etc., but considerable washing is necessary to dislodge it.

Test Meals.—The Ewald-Boas test breakfast consists of white bread, 30 to 40 gms., and water or tea without sugar or cream, about 400 c.c. This should be taken at the time the patient is accustomed to breakfast. The bread should be masticated to a fine pulp and the whole ingested in not over 10 minutes. According to former methods the stomach tube was passed in just 1 hour from the completion of the meal and as much as possible of the gastric contents syphoned off. Normally one obtains from 30 to 70 c.c. of an acid fluid containing the partly digested bread in a granular condition and some mucus. If but little is obtained, as in cases of hypermotility, the meal was repeated on subsequent mornings varying the time before removal until confident that one is obtained while digestion is at its height. Unless from previous observation one is sure that the stomach empties itself well it should always be thoroughly washed out the night before a meal. The results of examination of the first test meal should be accepted with caution especially should they indicate disturbed motility or reduced acidity and the meal repeated until the patient has become accustomed to the procedure.

Since bread is so variable in its composition, Dock substitutes for it 1 shredded wheat biscuit.

Since the above breakfast consists so much of starch and water and contains so little proteid it cannot test well the most important of gastric digestive functions, that is, the hydrolysis of proteid.

Riegel proposed a meal consisting of 1 plate of beef soup, from 150 to 200 gms. of beefsteak and 150 gms. of mashed potatoes. This should be eaten at the patient's regular dinner hour and removed at the end of from 3 to 4 hours.

Fischer's meal consists of the ingredients of Ewald's test breakfast, plus a quarter of a pound of finely chopped, lean beef boiled and slightly seasoned. It is to be removed at the end of 3 hours.

The study of gastric conditions by the chemical analysis of the stomach contents after a test meal has during the last 15 years fallen into almost complete disuse. The röntgenological examination is in part responsible for this. This will give certain data much more accurately than can the test meal and other data which the latter cannot give. And yet the röntgenological examination cannot replace entirely the chemical examination. One of the chief reasons why the latter has been so abandoned is that the profession has been careless in this work and so did not get convincing results. The introduction of the Rehfuss tube and the examination of specimens of the gastric contents removed each 15 or 20 minutes (see page 351) is a great advance in our methods and introduces a new era in gastric diagnosis.

Among the common mistakes which have been made in gastric analysis are the following:

The test breakfast should resemble as closely as possible the patient's customary meal. The Ewald breakfast is like the German early breakfast but does not at all resemble the American. It should be removed when digestion is at its height and this seldom is at the end of just 60 minutes.

It should be eaten at about the same hour that that particular patient is accustomed to eat a meal of that general character. In this country the convenience of doctor and nurse rather than the habit of the patient is too much considered. The stomach should be thoroughly washed out the night before since a crumb of food of the day before can completely vitiate the results.

The first meal is of practically no value, and often the second, because of the fear and disgust which the tube may inspire.

Fischer has shown, and this probably illustrates the difference between any typical starch and proteid meals, that the results with his meal are much more constant than those with the Ewald breakfast. For instance, the diagnosis made early using the Ewald breakfast had to be changed after later meals in 40% of the cases, while in but about 8% with his meal. If he used both meals in the same cases the results were similar in 67% of the cases while 18% of those who showed hyperacidity with the Ewald breakfast showed less with his meal and 15% more. Of the cases subacid with the Ewald breakfast 30% were normal by his. In general it may be said that the Ewald breakfast will give some idea of what the stomach will do with an indifferent meal which excites but little secretion, while the Riegel will show the gastric functional ability when a greater tax is made upon the secretory cells. Some stomachs can handle the test breakfast well but not the larger meal, while others respond well only to the greater stimulus. Fischer gives several points of differential diagnosis based on the use of 2 meals. If the stomach be subacid to the breakfast but normal after the proteid meal we may suspect that the secretory structures are normal but that the constant presence of foods due to atony has reduced the sensitiveness of the mucosa; if the gastric juice is subacid both to the proteid meal and to the breakfast, one may suspect organic changes; if subacid after the breakfast but hyperacid after a proteid meal, one might suspect defective innervation and the same would be true if it were hyperacid after the breakfast and normal after the largest meal. If the contents are hyperacid after the breakfast and still more so after the proteid meal one may suspect an increase of the oxyntic cells and especially so if the secretion continues for several hours after the meal. If the symptoms and an increased secretion both diminished after the meal, disturbed innervation may be suspected. Fischer emphasized the point that certain cases of dyspepsia, which for some time have been on an almost starvation diet, need not be fed up pretty well before a test meal is given. This may cause a gastric upset, but the flare-up of the condition will be an advantage in the diagnosis.

A point of importance in neurotic cases is that the time at which the meal is given should be chosen with reference to the symptoms, since at other times than during the nervous disturbances the gastric condition may be found normal.

Acidity of the Gastric Juice.—The gastric contents to be examined should be tested first with litmus. This will indicate its reaction in general. If acid, this may be due to hydrochloric acid, free or bound, to organic acids and to acid-salts. In the great majority of cases the litmus will turn red; in a very few cases the fluid is alkaline.

The fluid should next be tested for the presence of free hydrochloric acid.

The gastric juice of the normal person is strongly acid from the presence of hydrochloric acid and certain acid-salts. This acid at the height of digestion after a mixed meal is present in 2 conditions: the bound and the free. By bound acid we mean acid which has entered into a loose combination with certain organic bodies without disassociation of the acid-molecule and which in all ordinary chemical reactions, with the exception of certain color reactions, behaves as HCl. It will react acid to litmus and phenolphthalein but neutral to certain other delicate color reagents as Günzburg's reagent, Congo-red, dimethylamidoazobenzol, etc. These acid-binding bodies in the stomach are proteins and many of its split products, especially the hexone bases, and mucus.

There are in the gastric secretion also certain alkalies as sodium hydroxide and ammonia. These form with the acid neutral chlorides and take no further part in the gastric acidity.

The normal stomach has a remarkable regulating mechanism which so controls its acidity that although there are great variations in the total amount of acid secreted, depending on the meal to be digested, the qualitative relations of the fluid are quite constant for the same person at the same time after meals of similar composition. How remarkably accurate is this control is shown better by the constant differences which follow changes in the composition of the meals.

The molecule of bound acid (which need not be hydrochloric) is necessary if pepsin is to split the molecule with which that acid is bound. Theoretically an optimum digestion could be attained were there only just enough acid present to saturate all the acid-binding bodies, but the mucosa actually does maintain in the stomach during digestion a fairly constant excess of acid, that is, of hydrochloric acid, which is free since in excess of the acid-binding bodies. This free acid will give certain color reactions which the bound does not. This free HCl probably has an important function to play in sterilizing the gastric contents, in controlling gastric mobility and pyloric spasm and in the formation of hormones to stimulate the activity of other organs of digestion.

In conditions of diminished secretion of hydrochloric acid the gastric juice may contain large amounts of organic (especially lactic) acid which also may aid the pepsin to split the protein molecule.

The tests for free hydrochloric acid are for the most part color-tests for any free acid whether mineral or organic.

Methyl violet is the indicator suggested by v. d. Velden, who first showed the presence of free HCl in the gastric juice.[3] This is still a very satisfactory reagent. One drop of the saturated aqueous or alcoholic solution of methyl violet is added to a test tube half full of water and then diluted with more water until it has a pale violet color. This fluid is then divided in 2 test-tubes; to the one is added the filtered gastric juice, to the other the same amount of water. Free HCl will turn the violet to a fine blue color. This indicates 0.025% of free mineral acid; to produce the same reaction would require a much larger amount of free organic acid.

Tropeolin oo has been used, but is less sensitive than the above, indicating as it does but 0.03% of free HCl. The test is made in the same way as the above; free HCl will turn the yellow to a reddish-yellow color.

The test easiest to use is *Congo-red paper* which a free mineral acid will turn to a sharp blue color, while free organic acids, even in strong concentration, will give a much less definite shade of blue. Acid salts, if strong, would give a positive test but not in the concentration found in the stomach.

Dimethylamidoazobenzol is the reagent now most commonly used. In the presence of free mineral acid the yellow color of its solution changes to a fine pink. It reacts also, however, to organic acids and acid phosphates in concentrations which might occur in the stomach.

Günzburg's solution (phloroglucin, 2; vanillin, 1; alcohol, 30) is the best test since it reacts only to free mineral acids and in gastric juice, therefore, only to free hydrochloric acid. One or 2 drops of this solution (which should be kept in a tightly corked blue bottle and not allowed to get too old) are gently warmed on a porcelain dish until just dry. One drop of the gastric juice is then allowed to come into contact with this and the gentle warming continued. If free acid is present a beautiful crimson line will appear at the edge of contact. This is a fairly sensitive test, less sensitive than the others, but of more value since it is final.

It is to be emphasized that the above color-tests all indicate free acid, the most of them free mineral acid, and so free hydrochloric acid; that is, acid in excess of all acid binding bodies such as proteids, hexone bases, etc. Enough acid will have been secreted so that some will be free after a carbohydrate meal in from ½ to ¾ of an hour; after meat, in from 1 to 1½ hours; and after milk and potatoes, in ¾ of an hour.

Total Acidity.—The estimation of the total acidity of the stomach contents is the starting-point in all gastric analysis. This figure represents the amount of hydrochloric acid which would be present were all the acidity in the stomach at that particular time due to this acid; this, compared with the amount of free acid, gives a good picture of the secretory and the motor ability of the stomach. The total acidity is the sum of the free and the bound hydrochloric acid, of other acids, as lactic, butyric, etc., and of all acid-salts, *e.g.* (phosphates) present at that time.

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[3] Deutsch. Arch. f. klin. Med., Bd. 23.

To 10 c.c. of gastric juice is added an indicator sensitive to all acid reacting substances. Tenth-normal NaOH is then added slowly from a buret, stirring all the time until the change of color shows throughout the whole volume of fluid. This titration may be done in a porcelain dish, a beaker, or an Erlenmeyer flask, the latter 2 against a white background.

The indicator usually used is phenolphthalein, 2 or 3 drops of a 0.5% alcoholic solution. Among the others used are litmus, cochineal, methyl orange, etc. Phenolphthalein is preferred because of the sharpness of its end reaction. It is colorless in acid and brilliant red in alkaline solution. Yet it is perhaps the most unfortunate choice of all since it is not accurate in the presence of ammonia salts of which there is a fairly large amount in the gastric contents. The results of the titration will therefore be too high. The reason for its continued use is the desire to get comparable results to which an empirical value may be given.

The unfiltered gastric juice, shaken well to a homogeneous suspension, should be used since the solid particles contain relatively more of the acid than do the fluid portions.

The acidity of the gastric contents may be expressed in 2 different ways. If the number of cubic centimeters of $0.1N$ NaOH used be multiplied by 0.00365 gm. we would have the weight of acid measured as hydrochloric acid neutralized, yet it is never the case that all the acidity is due to HCl.

TABLE OF EQUIVALENTS

Acidity per cent.	Gravimetric per cent.
10	0.0365
14	0.05
20	0.073
27	0.1
34	0.125
40	0.146
48	0.175
50	0.182
55	0.2
61	0.225
70	0.25
73	0.275
80	0.292
87	0.317
90	0.329
95	0.347
100	0.365
105	0.383
109	0.4

The better and usual method is to follow the suggestion of Jaworski who introduced the term *acidity per cent.* (abbreviation of this, A. P.) for the number of cubic centimeters of the alkali which would be required to neutralize 100 c.c. of the gastric juice. Since 10 c.c. of stomach contents is the amount used the titration figure multiplied by 10 will give the acidity per cent. without reference to the character of acid bodies which may be present.

An illustration: if, using phenolphthalein as indicator, 10 c.c. of the gastric contents required 8 c.c. of $0.1N$ NaOH to neutralize the acids present, the acidity per cent. would be 80 A. P. Supposing that HCl were the only acid present, then the gastric contents would contain 0.29% HCl. To avoid confusion, the symbol of gravimetric percentage is never used for "acidity per cent."

Free Hydrochloric Acid, Mintz Method.—Ten cubic centimeters of the gastric contents are titrated with $0.1N$ NaOH until the test for free acid is no longer positive. This method assumes that the NaOH will neutralize the free before the bound HCl.

Of indicators used undoubtedly the most accurate is Günzburg's. As the sodium hydroxide is added, small drops of the stirred fluid are removed by a glass rod, or better still, a platinum oesa, and tested on a porcelain dish (see page 345). Fleiner would add 25 to 30 drops of the Günzburg reagent directly to the gastric contents and then, as the sodium hydroxide is added, removes small drops which he warms in a porcelain spoon. Sahli, who also adds from 25 to 30 drops directly to the fluid, recommends that the glass rods with which the alkali is mixed with the gastric contents themselves be warmed, for the crimson color can be seen on the rod. Since a certain amount of gastric juice is lost in each of the Günzburg tests the results should be confirmed using a fresh portion from which fewer drops need be removed.

A much easier method, and one used in many clinics, is to touch the stirring rod after the addition of each fresh installment of alkali to a strip of Congo-red paper and to add the alkali until this no longer turns blue. Some to save time find the end reaction approximately with Congo-red, and then more accurately with Günzburg's reagent. The easiest and most popular method of all employs as indicator a very small drop, the little which clings to the end of a glass rod, of dimethylamidoazobenzol. This in the presence of free acid takes a bright red color. The sodium hydroxide is added until the red just disappears. A drop of phenolphthalein is then added and the titration continued to determine the total acidity.

The results with these 3 indicators are by no means the same. Those with Günzburg's will always be the lowest and those with dimethylamidoazobenzol usually the highest. Those with Congo-red paper vary, but will stand between those of the other 2.

Töpfer's Method.—This method now is chiefly historical. The indicators used in the titration are dimethylamidoazobenzol for the free hydrochloric acid and then a 1% aqueous solution of alizarin for the bound hydrochloric acid. The latter indicator, however, has been abandoned, and the most accurate way of estimating the bound acid is to subtract the free from the total acidity.

Hydrochloric Acid Deficit.—In cases with an insufficient gastric secretion of HCl it may be desirable to determine the acid deficit; that is, the amount of HCl necessary to saturate the acid-binding bodies present in the gastric contents. This is done by adding *0.1N* HCl to 10 c.c. of the gastric contents until the test for free acid is positive. The amount necessary to add will depend on the amount of bound HCl already present, the amount of proteid and bases which can bind the acid, and the amount of alkali secreted; hence a better term than HCl deficit is that suggested by Sahli, "saturation deficit." Congo-red paper or Günzburg's reagent can be used, but the former is sufficiently delicate. The determination of this value aids in following the progress of a case and in evaluating a therapy.

Total Hydrochloric Acid.—The hydrochloric acid secreted by the gastric glands may be present in the gastric contents as free acid, bound acid, and as neutral chlorides. The total neutral chlorides includes those from the food, those formed in the stomach by the reaction of an alkali and the acid and those chlorides secreted as such.

By " total hydrochloric acid " of gastric contents is understood the sum of the bound and free HCl (" the physiologically active hydrochloric acid ") although some of the neutral chlorides were formed from the HCl secreted as the acid. The Lütke-Martius method for determining this total HCl is based on the principle that the difference between the total chlorine (*a*) and the chlorine left after incineration (*b*) represents the HCl that was volatilized by heat. This method has been corrected by Reissner, who showed that NH_4Cl also will be volatilized. He, therefore, first neutralizes the gastric juice with *0.1N* NaOH using litmus as indicator. This neutralized fluid is then ashed and the chlorine determined $\acute{a}$:

$$a-b=\mathrm{HCl}+\mathrm{NH_4Cl},$$
$$a-\acute{a}=\mathrm{NH_4Cl},$$
$$\acute{a}-b=\mathrm{HCl}.$$

The Arnold and Lütke methods are used in these determinations (see page 131).

Determination of " *a*."—Ten cubic centimeters of the gastric fluid are measured with a pipet into a 100 c.c. measuring flask. Twenty cubic centimeters of Solution 1 are added, the mixture stirred and allowed to stand for 10 minutes. A few drops of 8% $KMnO_4$ are then added if necessary to decolorize the fluid. The flask is then filled with water to the 100 c.c. mark and the contents well mixed. This is then filtered through a dry filter until over ½ has passed through. Fifty cubic centimeters of this filtrate are measured into a beaker and Solution 2 then added from a buret until the resulting brown color is permanent. The number of cubic centimeters of Solution 2 necessary to precipitate the excess of silver are then multiplied by 2, since but half the filtrate was used in this titration. This product, subtracted from the amount of $AgNO_3$ originally added, will give the amount of $AgNO_3$ which was necessary to precipitate the chlorine.

Determination of "*b*." —Ten cubic centimeters of the gastric juice are evaporated to dryness on a water-bath in a platinum dish. The residue is then burned over the free flame until the ash no longer burns with a luminous flame. It is not brought to a red heat, since this would volatilize some of the chlorides. The ash is then rubbed up well with water using a glass rod, extracted with about 100 c.c. of warm water, brought onto the filter and washed until a few drops of the filtrate no longer give a precipitate with $AgNO_3$ To the whole filtrate are then added 10 c.c. of Solution 1, and the determination continued as for " *a*."

Determination of "$\acute{a}$."—Another 10 c.c. are first neutralized with *0.1N* NaOH, using litmus as indicator, then ashed and the remaining chlorides determined as for " *b*."

$(\acute{a}-b)\times 0.0365$ gm. = the per cent. of HCl.

Absolute Amount of Hydrochloric Acid Secreted.—The preceding methods are intended to give merely the percentage of the acid in the stomach contents at any one time. It is sometimes desirable to determine the total amount of acid present at a stated time. This method has some scientific, but no practical, value since considerable of the acid secreted will have already passed into the intestine.

To determine this the stomach-tube is introduced and as much of the gastric juice as possible is syphoned out. Then 300 c.c. of water are allowed to flow in and out of the stomach several times. From the difference in the specific gravities of these 2 fractions the amount of gastric juice in the second fraction can be computed. The acid of the first fraction is then determined and then that of the second calculated and added to it.

Physiology of the Gastric Juice.—After the ingestion of a test meal the secretion of gastric juice begins almost immediately. The hydrochloric acid will at first be bound as soon as secreted but by the end of a half-hour after the test breakfast or 2 hours after the Riegel meal enough will have been secreted that some will remain free. The amount of acid rises to a maximum where it remains until the products of digestion pass on into the intestine. Then the acidity will begin to fall. The gastric juice as it emerges from the gastric glands would seem to contain about 0.5% HCl. This is at once partly neutralized by the other constituents of the juice so that 1 hour after the Ewald breakfast the total acidity normally averages from 40 to 60 A. P. of 0.15% to 0.22% HCl. Lactic acid is not present and phosphates are not present in any important amount. Of this total acidity the free HCl will vary from 20 to 60 A. P. (from 0.05% to 0.2%) and the bound from 0.012% to 0.11%. With the Riegel meal the normal total acidity is about 75 A. P. (from 90 to 100 A. P.) and the free about 44 A. P.

An acidity per cent. over 70 (0.25% HCl) has been considered hyperacidity. This is far from the truth. The acidity of the normal stomach is sometimes 0.33% (A. P. 90) or over and the person quite free from gastric discomfort of any kind. We were able to determine this from the analysis by our medical students of their own gastric contents which at one time we required them to make. "Hyperacidity" is not a question of the percentage of HCl in the gastric juice, but of the contraction of the pylorus which leads to slight retroperistalsis and this causes the symptoms interpreted as "hyperacidity" (pyrosis, acid eructations, etc.) which may be present when the acidity per cent. is normal or slightly low.

The Value of the Tests for Acidity of the Stomach.—Of all the above tests that for the presence of free HCl is the most important. A gastric juice which contains a normal amount of free HCl will quite certainly be normal in other ways, since the secretion of this acid is the first gastric function to suffer in any disturbance of this mucosa. While practically every stomach can secrete some HCl the normal organ will always provide an excess, that is, some free HCl. The quantitative determination of the total acidity and of the free HCl is of greatest value not in diagnosis but in following the progress of our therapy. Very accurate work is clinically of little value since the total amount of secretion can never be recovered.

If a bread and water meal is used the phosphates may be disregarded. If free HCl is present organic acids may be disregarded (provided the stomach was clean before the meal and none present in the food). If the total acidity is high and no free HCl present, the acidity is due for the most part to organic acids. This may be confirmed by the lactic acid test or the odor of the other organic acids. Many bacteria will be present. If free hydrochloric is present and the total acidity low, the acidity will be due to hydrochloric acid and the motility of the stomach may be assumed to be good since the acid-binding bodies have passed on into the duodenum. If, on the other hand, the total acidity be moderate and free acid small in amount a poor motility may be assumed with the retention of the products of digestion.

A review of past work using Ewald's breakfast and 1 specimen of gastric contents is given by Sahli as follows: (*A*) There is normal acid secretion: (1) Often in ulcer of the stomach and stenosis due to the contraction of its scar; (2) in gastric neuroses; and (3) in simple atony.

(*B*) Hydrochloric acid is over 0.2% (A. P. 55) and the total more than 70 A. P. (it may reach 0.35% and very rarely 0.8%): (1) In the majority of cases of ulcer of the stomach; (2) in true continuous hypersecretion (but not the hypersecretion due to motor stasis); (3) in simple hyperacidity and hypersecretion during digestion, at which time the per cent. of acid may be abnormally high; (4) in paroxysmal hypersecretion (gastroxynsis) in neurotic individuals, who, following some excitement or other disturbance, vomit large amounts of acid juice; (5) in some cases of chlorosis (in 22 of 30 of Riegel's cases); (6) in early stages of chronic gastric catarrh; and (7) often in insanity.

(*C*) The secretion of hydrochloric acid is diminished in (1) fevers; (2) in severe anemias; (3) in the majority of cases of chronic gastric catarrh; (4) in many gastric disorders due to general neuroses; (5) in many forms of mental diseases; (6) after long-standing jaundice; (7) in many chronic cachexias, as tuberculosis of the lung, but not always; (8) in chronic passive congestion due to heart disease or to emphysema, etc.; (9) sometimes in chronic nephritis; (10) after the long use of alkaline and saline purges; (11) as a "fatigue" symptom following periods of hypersecretion.[4]

(*D*) Free hydrochloric acid is absent on repeated examinations (and yet the stomach always contains a certain amount of this acid bound) in all conditions under C of a severe grade (especially in amyloid disease of the stomach, toxic gastritis, nervous dyspepsia, phthisis, and cardiac disease). Its absence is most important in (1) severe febrile diseases, particularly the infections; (2) gastric carcinoma (also other carcinomata); (3) atrophic gastric catarrh; (4) pernicious anemia. The most important of these is gastric carcinoma.

Standards vary with nationalities, with classes of society and still more with individuals. Strauss, at Giessen, thought 68 a fair average total acidity; at Berlin 47. In this country we use an unusual meal and must judge the patient not according to any physiological normal, but to an empirical standard gained from the examination of many cases.

Among 526 cases of the Johns Hopkins Hospital clinic whose records were studied were the following. In all cases the Ewald breakfast was used.

Pernicious Anemia, 13 Cases.—Amount removed, 10 to 80 c.c. All were subacid, the highest total acidity being 38 (A. P.) and below 10 A. P. in 10 cases. In only 1 was there any free HCl. In 2 the fluid was neutral to litmus; in 1, alkaline. Lactic acid was present in 2 cases (in 1 of these the diagnosis was confirmed at autopsy, in the other, not). In 2 cases of severe secondary anemia the fluid was only slightly subacid and free HCl was present in both.

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[4] Foster and Lambert, Jour. of Exp. Med., 1908, vol. x, No. 6, p. 820.

Malignant Disease not of the Stomach.—Of these cases, 10 were carcinomata, and 4 sarcomata. All were subacid (total acidity less than 40). Of the carcinoma cases free HCl was present in 7, was absent in 2 and the fluid neutral to litmus in 1. Of the sarcoma cases in none of the 4 was any free HCl present.

Catarrhal Jaundice, 9 Cases.—The fluid removed varied in amount from 10 to 86 c.c.; total acidity from 10 to 70 A. P. In 3 cases no free HCl was present; in 1 the fluid was alkaline to litmus; in none was lactic acid found. In a few cases the acidity progressively diminished during the course of the disease.

Cholelithiasis, 14 Cases.—Amount removed, 5 to 120 c.c. There was hyperacidity in 1 case (total 79 and 82 on 2 examinations, free HCl, 42 and 49 respectively); normal acidity (40 to 70) in 6 cases; below 40 in 6. In 4 of these 6 there was no free HCl. Lactic acid was demonstrated in 1.

Cirrhosis of Liver, 6 Cases.—Normal acidity was present in 1, subacidity in 5. In 4 of the 6 cases no free hydrochloric acid was present. Of these 6 in 1 the gastric juice was practically neutral, while that of another of these cases contained lactic acid.

Tuberculosis of Lungs, 10 Cases.—The total acidity was normal in 3 of these 10 cases and subacid in 7. In 3 there was no free acid. Of these 3 the fluid was neutral in 1 and 1 contained lactic acid.

Intestinal Troubles.—Diarrhea, 9 Cases.—In 4 the acidity was normal, in 4 subacid and in 1 almost neutral. In 4 no free acid was present; 1 contained lactic acid. *Constipation*, 5 cases, of which 2 had normal acidity, 3 were subacid, and in 1 there was no free hydrochloric acid. *Colitis*, 2 cases, both subacid and without free acid, and both containing lactic acid. *Amebic dysentery*, 1, which was hyperacid (92 total and 83 free).

Arteriosclerosis and Cardiac Diseases, 17 Cases.—Of these 8 were normal, 6 subnormal and 3 without free acid. One of these was almost neutral.

In a group of 36 cases of *miscellaneous diseases*, 25 showed normal gastric conditions. Subacidity without any free acid was present in cases of heat prostration, of enteroptosis, chronic bronchitis, peripheral neuritis (with lactic acid), chronic nephritis, bronchopneumonia, and malaria.

Fractional Determination of Gastric Secretion.—The inaccuracies inherent in the usual method of examining the gastric contents removed just once and therefore seldom at the height of digestion, have led to the development by Rehfuss [5] of a technic using a modification of Einhorn's duodenal tube which makes it possible to examine several specimens removed at intervals during the digestion of a single meal. The test meal consists of 40 gms. of water crackers and 10 oz. of water, 2 of which are reserved to be swallowed with the tube. The patient is allowed about 10 minutes to consume this meal and then immediately swallows the tube together with the 2 oz. of water. The patient should be careful not to swallow the saliva which forms rapidly during the next few minutes. The patient is made as comfortable as possible, is urged to read, etc., and suffers little inconvenience with the tube in his mouth. At 20-minute intervals from 15 to 25 c.c. of the stomach contents are aspirated by gentle suction until no more can be obtained when the patient is in the dorsal, right and left lateral and knee-chest postures. Each fraction is examined separately as regards free and total acidity and the presence of pepsin.

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[5] Am. Jour. Med. Sci., June, 1914, vol. 147, p. 848; Jour. A. M. A., Sept. 12, 1914, p. 909.

Fishbaugh[6] using Rehfuss' method calls attention to the curves of gastric secretion which the cases studied by this method present. In 1 group of cases the curves of the secretions reach their maximum in 91 minutes (average) and fall towards the end of gastric digestion. This would seem at first thought to be the normal condition. The need of further secretion past the stimulus lessens, the juice is less concentrated, while some of the acid is neutralized by the products of digestion. In a second group the curves are still rising at the end of gastric digestion (average 127 minutes) so that the last few cubic centimeters of gastric juice are the most concentrated of all. This is normal in some healthy individuals. In the third group of cases with gastric secretion absent or delayed there

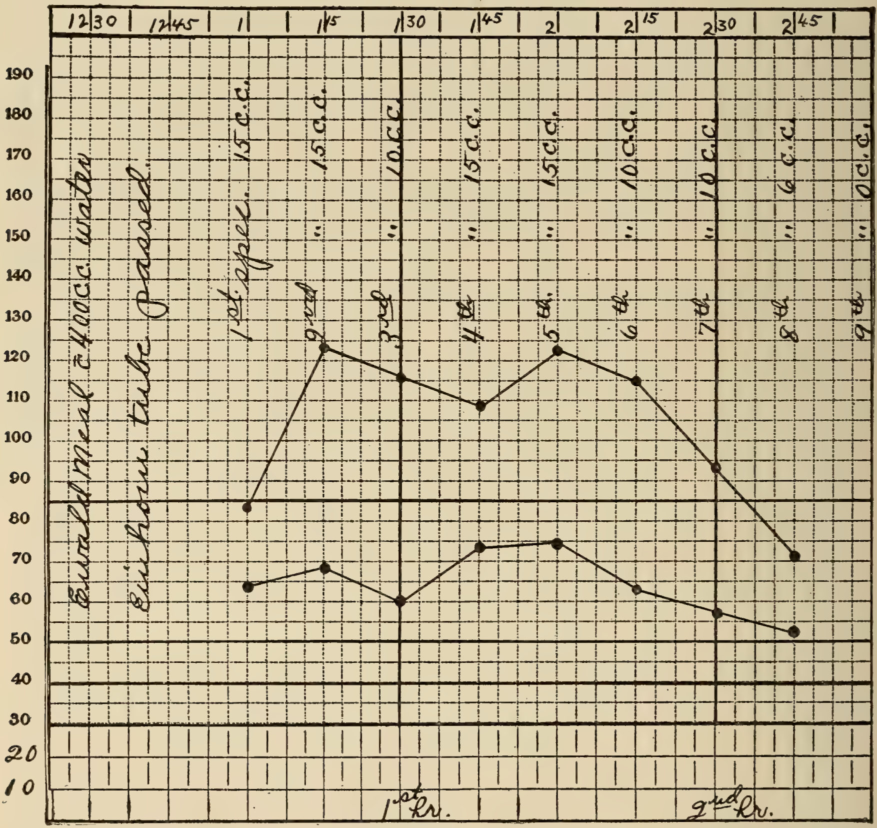

CHART I.—M. H., Duodenal ulcer. Ewald breakfast given November 22, 1920, without previous lavage and removed through a Rehfuss tube at 15 minute intervals. That evening the stomach was well washed out and the breakfast repeated. See Chart II. The tube had not entered the duodenum in 3 hours.

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[6] The Jour. of A. M. A., Oct. 28, 1916, vol. lxvii, p. 1275.

may be an absence of acid and enzymes, or an absence of acid with enzymes present, or the acid and enzymes may appear late.

To illustrate this method and also to emphasize some of the mistakes inherent in clinical gastric analysis we give 2 charts of examinations made on 1 patient on successive days. The patient, a man 41 years old, is a clear case (the clinical history and especially the röntgenological examination positive for this) of duodenal ulcer with considerable retention. A test was made in the afternoon of Nov. 22, 1920. Chart I. The meal was given at 12.30 P.M. and 8 specimens removed at 15-minute intervals beginning at 1.00 P. M. The total acidity was high reaching 124, the free acidity reached 74 while the bound acidity was very high, even from 50 to 64. This shows that there was still considerable food in the stomach before the meal was given.

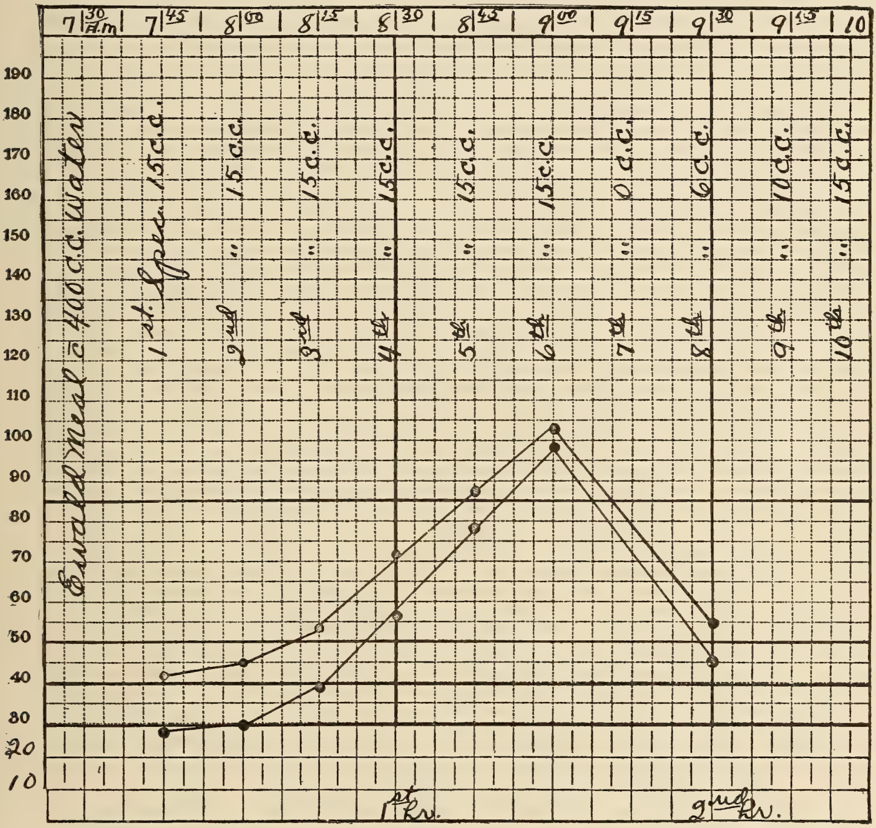

CHART II.—M. H., November 23, 1920. Ewald breakfast given at 7.30 A.M. and removed at 15-minute intervals. The stomach had been well washed the evening before. Compare with Chart I. Between 9.30 and 9.45 the tube entered the duodenum.

That night the stomach was well washed out and the same meal given at 7.30 A.M. the following morning. Chart II. In 1 hour the free acidity was 56 and the total 72. According to former methods therefore this would pass as a case of normal acidity. Thirty minutes later however the free was 94 and the total 104.

The former methods of examination (removal of the meal in 60 minutes) led to frequent error in the cases of the first 2 groups, but in the third to

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very frequent and serious errors in that these cases were usually diagnosed "achylia gastrica" which is seldom the true condition. It is possible that in those cases with late secretion the mucosa is fairly normal but responds only to the chemical stimulus, the psychic being absent or ineffectual.

Undoubtedly the work of Rehfuss and his co-workers is one of the great advances in clinical medicine. The results of gastric chemistry had been so unsatisfactory that the diagnosis of our gastric cases has been left almost entirely to the röntgenological departments, although in the very nature of the case their aid, while valuable, is of necessity limited and most of the evidence they obtain indirect.

Sahli's Desmoid Reaction[7] is based on the assumption that catgut in its raw state is soluble in gastric juice, but indigestible in pancreatic juice.

A pill of methylene blue, 0.05 gm., or of iodoform, 0.1 gm., or of both, and sufficient ext. glycyrrhizæ to make a mass not over 3 or 4 mm. in diameter is enclosed not too tightly in a square piece of thin rubber dam the twisted neck of which is tied with 3 turns of raw catgut, number 00, previously soaked until soft in cold water. Both knots should be on the same side of the bag. The free edge of the rubber should extend about 3 mm. beyond the ligature, and its edges should not cohere. The completed pill should sink instantly in water and should be proven to be water-tight.

This "desmoid pill" is given with or just after the midday meal and the urine, collected at intervals of 5, 7, and 18–20 hours, examined for the methylene blue (or iodine, or both). To demonstrate the methylene blue it may be necessary to boil the urine with ⅕ volume of glacial acetic acid. The iodine is recognized by the rose color which develops when pure nitric or sulphuric acid is added and the urine then shaken out with a little chloroform. If these indicators are found within from 18 to 20 hours after the pill is swallowed the test is positive; otherwise, negative.

It is claimed that this test is simple, comfortable for the patient, and that it will test the digestive (both HCl and pepsin) activity of the stomach at the height of digestion of a full meal and therefore sometimes indicates good functional activity when the test breakfast shows no free HCl. It is useful therefore to distinguish between true achylia, as in carcinoma or pernicious anemia, and that present with less serious disorders in which the stimulus of the Ewald breakfast is insufficient.

The most important result obtained thus far is that in no case of cancer of the stomach or of pernicious anemia in which the test breakfast showed an absence of free HCl did the capsule open. This test has a real though limited value, but it cannot entirely replace the stomach-tube and test meal, or the X-ray.

Various other methods of testing the gastric juice without using a tube have been proposed, especially by means of a hollow capsule containing reagents. The best of these was described by Rehfuss.[8]

The ferments of the gastric juice are pepsin, rennin and lipase. Riegel believed that much of the attention which has been directed to gastric acidity should be turned to the study of gastric ferments.

PEPSIN.—The qualitative determination of pepsin need never be made when there is any free hydrochloric acid present, since the pepsin-forming function of the stomach is more resistant in disease than is the hydrochloric-acid-forming function. (The water-secreting function is the most resistant of all.) The determination of pepsin is valuable when there is no free HCl, as in carcinoma, pernicious anemia and atrophic gastric catarrh. Schiff found that in cases with marked hypacidity and anacidity due to benign causes the amount of pepsin secreted is little changed, but that in slight hypacidity due to gastric cancer it may be greatly reduced.

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[7] Sahli, Corresp. Bl. f. Schweiz. Ärtzte, 1905; Boggs, Johns Hopkins Hosp. Bull., 1906, vol. xvii, p. 313; Carey, Boston Med. and Surg. Jour., May 2, 1907, vol. clvi, p. 563.

[8] Am. J. Med. Sci., June, 1914.

Qualitative Determination.—The presence of pepsin is assumed if the gastric juice (HCl added if none be free) will digest egg albumin or fibrin. The fibrin is prepared as follows: Fresh ox-blood is whipped and the fibrin kept in running water until perfectly colorless. It is then cut in fragments of equal size which are put for a few days in alcohol and then for 1 or 2 days in a cool, concentrated, neutral, carmine solution until fully stained. It is then well washed, pressed out and kept in glycerin stained with carmine. Before use the fibrin is well washed with water to remove all the glycerin. To prepare egg albumin the egg is boiled for 5 minutes, not longer, and the white then cut with an ordinary cork-borer into 5 mm. cylinders and these into disks 1 mm. thick. These disks are kept in glycerin and are well washed in water before they are used.

To the fluid to be tested for pepsin hydrochloric acid is added if necessary until some is free, then the fibrin or egg disks are added and the specimen put in a thermostat. If pepsin is present the fibrin will show signs of digestion first by the liberation of the carmine in from 15 to 30 minutes, the egg albumin first by the rounding of the disks' edges in from ½ to 4 hours.

Sahli recommends that both albumin and fibrin be tried, since some gastric juices can digest the fibrin easily but not the albumin, and that the tubes be kept at room temperature in order that the difference in them may be more clearly observed.

Quantitative Determination.—The general law of ferment action is that activity varies approximately as the square root of the amount of the ferment present.

Mett's quantitative method of estimating the amount of pepsin is fairly accurate if the gastric fluid has been diluted 16 times. The fresh egg albumin is filtered and exposed to a vacuum produced by a suction pump for several hours in order to remove all gas. A beaker is then filled with the albumin, a bundle of glass tubes, each about 1 cm. long and 1 to 2 mm. wide, are then immersed in it and the beaker heated for 5 minutes in water at 95° C. The tubes are then carefully removed, their outside cleaned, and both ends closed with sealing-wax. One cubic centimeter of the filtered gastric juice is mixed with 15 c.c. of *0.05N* HCl and well shaken. Pieces of the tubes full of albumin 2 cm. long remain in this fluid for 24 hours in the thermostat. The average distance to which digestion has progressed into the tubes is then measured. The square of this multiplied by 16 will give the units of pepsin in the gastric juice. (By unit is meant the amount of pure pepsin which in 24 hours will digest an average of 1 mm. of the albumin in several tubes.) The distance to which theoretically pure pepsin would progress into the tube is 4 mm.

THE FAT-SPLITTING FERMENT—*Volhardt's Method.*—The yolk of 1 egg is mixed with 30 to 40 c.c. of water; 10 c.c. of this mixture are added to the gastric juice, both fluids having been warmed separately in the thermostat. The mixture is then kept for 2 hours in a thermostat which registers from 37° C. to 40° C., then cooled. Ether, 75 c.c., and a few cubic centimeters of alcohol are then added and the specimen well shaken. A measured amount of the fat-containing ether extract thus obtained is now mixed with 50 c.c. of neutral alcohol and titrated with $0.1N$ NaOH using phenolphthalein as indicator, to determine the amount of fatty acid split from neutral fat by the gastric lipase. One next adds 10 c.c. of $0.1\ N$ NaOH and places the specimen on a water-bath for 2 hours in a flask connected with a condenser and a calcium oxide tube to exclude CO_2 (or it is allowed to stand for 24 hours in a closed flask at room temperature) to saponify the unsplit fat. Ten cubic centimeters of $0.1N$ HCl are now added to free the fatty acid and the mixture is again titrated with $0.1N$ NaOH, phenolphthalein as indicator, to determine the fat which was unchanged by the ferment while the specimen was in the thermostat. From the relation of these values the percentage of neutral fat which was split by the ferment is reckoned, and hence the number of units of ferments present, using Stade's formula, $p = \sqrt{f} \times \sqrt{t}$, in which p equals the product of digestion, f the units of ferment, and t the time. If "f" represents the amount of ferment which will split 1% of the fat in 1 hour, then, if after 3 hours one finds 6% split, 12 units of ferment must have been present.

Volhardt found that in the stomach in 2 hours from 30 to 36% of the neutral fat is split. The fatty acids thus liberated and later dissolved in the bile evidently aid in the emulsion of the neutral fats of the foods. It is possible therefore that the stomach does considerable of the work usually attributed to the pancreas.

It has been found that in hypochylia and achylia the lipase either is diminished in amount or is absent.

RENNIN.—The presence of rennin in the gastric juice is proved if this secretion, after it has been neutralized, coagulates milk without change of reaction.

Riegel's method of determining the presence of rennin is to mix from 5 to 10 c.c. of gastric juice (neutralized with $0.1N$ NaOH) with from 5 to 10 c.c. of fresh milk and to place this mixture in a thermostat. If rennin is present coagulation will be evident in from 10 to 15 minutes. If a longer time is required one should exclude curdling by some lactic acid which has been formed.

A *quantitative determination* of rennin may be made by mixing, in a series of tubes, equal volumes of fresh milk and various dilutions of the neutralized gastric juice and noting the highest dilution of the juice which will coagulate the milk. Boss found in normal cases this to be a dilution of 1 : 100 to 150. In cases of hypochylia the ability of the rennin to coagulate the milk may disappear when the juice is diluted 1 : 5 to 10. Glassner found that in some cases of pyloric cancer the rennin secretion is normal while that of pepsin is diminished, while in cancers of the fundus both rennin and pepsin are diminished. In general the variations in the amount of rennin run parallel to those of pepsin. Since rennin is the easier of the 2 ferments to estimate it may be that in time we shall follow its secretion rather than that of pepsin.

The Products of Gastric Protein Digestion.—We may divide the products of the peptic digestion of proteins into the following groups: albumoses, peptones and the products of further cleavage. The soluble albumin is precipitated by heat, the albumoses by the addition of an equal volume of saturated zinc sulphate and the peptone by phosphotungstic acid. The filtrate after this last precipitation will contain all products below the so-called peptone stage. Those various fractions of digestion-products are best determined by estimating the nitrogen in each filtrate, care being taken to reduce all quantities into terms of the original volume of the stomach contents obtained.

Benedict[9] advises to determine these precipitates volumetrically after centrifugalizing them to their smallest possible volumes. The meal used should contain but 1 proteid and this should be carefully weighed. We have used nutrose (a casein preparation) with good success. The nitrogen fractions of the contents of the fasting stomach of each patient studied should also be determined and these corrections made. The meals should be given at the same hour of the various days, and removed at the end of the same period of time.

From a theoretical point of view it is interesting that in cases of carcinoma of the stomach the digestion of proteid is so much more rapid than normal that one must assume the presence of an abnormal ferment.[10] Artificial digestion experiments using as ferment heated and unheated carcinoma tissue strengthen this assumption.

In our benign cases studied in this way the Ewald breakfast being used the average amount of nitrogen in albumose found was 51.7%; in the phosphotungstic acid precipitate, 31.4%; in the residue, 16.9%. In the carcinoma cases these figures were respectively 27.5, 47, and 27.6%.

Starch Digestion.—Undoubtedly the current idea that the saliva is not important in starch digestion and that the pancreas does it all must be corrected since from 50 to 70% of the starch of the food is rendered soluble in the stomach. The ptyalin digestion can continue throughout the stomach contents until the total HCl reaches 0.12% and within the food masses for a much longer time until the acid has penetrated to their center. Hence starch digestion is reduced in cases of hypersecretion and hyperacidity.

The stages of starch digestion are: soluble starch, erythrodextrin, achroödextrin and maltose. The relative amount of these may approximately be detected by the use of a very weak Lugol solution. The colors obtained vary from the blue to the blue-violet of the starches, the red to the mahogany-brown of the erythrodextrin while the iodine combination with achroödextrin is colorless. Since the later products of starch digestion have a greater affinity for the iodine than the earlier, 1 drop of weak Lugol's solution added to a small amount of gastric juice will give no blue color with the starch present if much achroödextrin also is there. On the other hand, if very little of the higher products are present the starch will give its blue color. From the number of drops of the Lugol solution which must be added before the blue color appears one may estimate approximately the extent of the starch digestion. (For a more accurate method see page 383.)

Lactic Acid.—The presence of lactic acid in the gastric contents is significant only when the food eaten contained none and no constituent from which it might easily be formed and provided the stomach had been well washed out the evening before. Riegel says its presence in the stomach in never normal except during sugar digestion. There are bacilli in the mouth which can produce lactic acid but not in the time allowed by a test meal. For this reason the safest test meal so far as lactic acid is concerned is Dock's which consists of water and a shredded wheat biscuit.

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[9] Am. Jour. Med. Sci., 1904, vol. cxxvii.

[10] Emerson, Deutsch. Arch. f. klin. Med., vol. lxxii, p. 415.

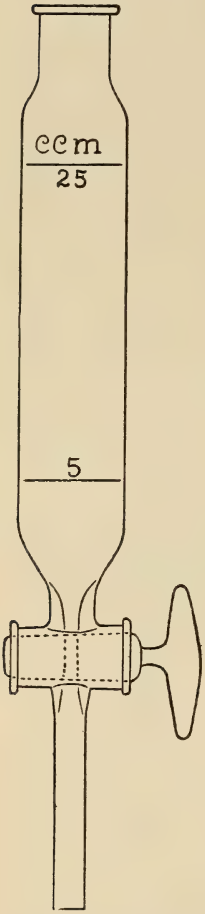

Fig. 70.—Strauss' separating funnel for lactic acid tests.

Uffelmann's test for lactic acid is the one in common use. To about 20 c.c. of 1% carbolic acid made up fresh each time in a test-tube is added 1 drop of 10% Fe_2Cl_6. A deep amethyst color is produced. Distilled water is now added until the blue color is so faint that one can see through the tube. This fluid is then divided in 2 test-tubes of equal diameter. To the 1 is added a drop or 2 of the gastric juice to be tested, to the other the same amount of distilled water. If lactic acid be present the amethyst color will change to a definite yellowish-green (canary-green) color.

The decolorization of the amethyst-colored fluid is not the test, but the development of a definite yellow. The blue is merely for contrast and so one may dispense with the carbolic acid. It is well to control the test with dilute lactic acid.

The test proposed by Strauss is better. In a small, specially marked separating funnel (see Fig. 70) one mixes 5 c.c. of the gastric juice and 20 c.c. of alcohol-free ether. One drop of hydrochloric acid is added to liberate any lactic acid which is bound to proteid and the tube well shaken to extract the lactic acid. The gastric juice is then allowed to run out and 5 c.c. of distilled water added to the ether extract, then 2 drops (from a medicine dropper to insure uniform size) of a 1% Fe_2Cl_6 solution. If at least 0.1% of lactic acid is present the water layer will take a definite canary-green color. This test is perhaps not as delicate as the other, but if it is positive it may be said confidently that a pathological amount of lactic acid is present. It is desirable to extract the gastric fluid with ether since a suggestive color may be given by sugar, proteid and alcohol and by some other inorganic acids (oxalic, citric, tartaric, etc.) while a positive test for lactic acid may be prevented if much phosphates or peptones are present. Again, ferric chloride may so cloud the gastric juice that the test will be obscured.

This test has been further modified by Knapp[11] who extracts 1 c.c. of gastric juice with 5 c.c. of ether. He then superimposes this extract upon a freshly prepared 1 : 2000 ferric chloride solution. Lactic acid will be indicated by a canary-colored ring.

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[11] New York Med. Jour., August, 1901.

De Jong[12] adds to 5 c.c. of the gastric juice 1 or 2 drops of HCl and evaporates this to a syrup over a free flame. He then extracts the residue with a little ether. The volume of extract is then made up to 5 c.c. with distilled water, 1 drop of 5% Fe_2Cl_6 added, and the whole well shaken. A definite green color is produced by 0.05% lactic acid.

Quantitative Determination of Lactic Acid.—For clinical purposes the Strauss modification of the Uffelmann method is an accurate enough estimation of the amount of lactic acid present since if that is positive the amount certainly is pathological and this is all we need to know.

Other Organic Acids.—Acetic, butyric, and valeric acids may be met with in the gastric contents and be recognized by their odor. They are the result of the bacterial decomposition of foods. Why in some cases one, in others, another, acid is formed is not known. One of our patients who gave all the symptoms of hyperacidity recently was given an Ewald test meal and at once complained of the taste of vinegar. The gastric contents were found rich in acetic acid.

Acetic acid carefully neutralized with soda if tested with 1 or 2 drops of Fe_2Cl_6 solution will give the bluish-red color of ferric acetate.

Butyric acid in the presence of fine fragments of $CaCl_2$ separates in fine oily droplets.

Total Organic Acid—Hehner-Maly Method.—The principle of the Hehner-Maly method for determining the total amount of organic acid in the gastric contents is that if a mixture of organic and inorganic acids is ashed the inorganic salts will remain neutral, while the organic will be changed to alkaline-reacting carbonates. The original acidity minus the final alkalinity may be considered to equal the mineral acidity.

Ten cubic centimeters of filtered gastric juice are neutralized with *0.1N* NaOH, using phenolphthalein as an indicator (amount necessary $=a$). This fluid is then evaporated and ashed, the ash taken up with water and titrated with *0.1N* HCl, phenolphthalein again used as indicator (amount necessary $=b$), $a-b=$ the mineral acidity ($=c$) then $a-c=$ organic acidity.

Bases of the Gastric Juice.—Sodium is the most important base of the gastric juice. Mehring has shown that the stomach secretes sodium carbonate to control the amount of hydrochloric acid. Reissner showed an increased secretion of alkali in gastric cancer. Ammonia also is present in considerable amount (normally 0.1 to 0.15 p.m.). Of all the tissues of the body which have been examined, the gastric wall contains the most ammonia. This throws considerable doubt on the accuracy of all titrations of gastric contents in which phenolphthalein was the indicator used (see page 346).

Fermentation.—Two kinds of fermentation may take place in the stomach, 1 with gas formation and 1 with lactic acid but no gas formation. Neither occurs unless considerable stasis is present. The former is the rule if free HCl is present, the latter when this free acid is much diminished in amount or is absent.

Lactic acid fermentation is proved by the presence of this acid in the gastric contents provided foods can be excluded as the source of the acid. Small amounts may be found when there is no genuine stasis as after a heavy meal or if there are pockets or little clefts in the stomach wall.

To test for gas fermentation 2 fermentation tubes should be filled with the well shaken unfiltered gastric contents. To 1 of these tubes, which serves as a control, is added a little glucose (since all the carbohydrate of the material tested may already have been fermented). The tubes are then left in the thermostat for 24 hours (some say for 3 or 4 days) at the end of which time the presence of gas, if any, is noted. Both carbohydrates and proteids may ferment in the stomach. This test is of some value in determining the degree of gastric stagnation; that is, the abundance of the gastric flora. The organisms of fermentation which are found in the stomach are yeasts, sarcinæ and long bacilli. Sarcinæ may be present in cases of high acidity, bacilli in cases of low acidity and of anacidity. Some believe that the presence of sarcinæ in cases of low acidity, or of long bacilli in cases of reduced acidity indicate cancer of the stomach. Yeasts are common in gastric contents. Coyon doubts that sarcinæ explain much of the fermentation, but he has isolated 2 bacilli, the enterococcus and Coccus radiaire, which he thinks are more important.

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[12] Arch. f. Verdauungskrankheiten, Bd. 2.

Among the products of fermentation have been found formic, lactic, and acetic acids, the higher fatty acids, ethyl alcohol, aldehyde, ammonia, hydrogen disulphide, and carbon dioxide.

HYDROGEN DISULPHIDE, from the decomposition of proteid, is rarely found in the stomachs of patients with malignant, but frequently in the stomachs of patients with benign, stenosis of the pylorus. The saliva and some foods, as radishes and onions, must be excluded as its source. This gas may be recognized by its odor, or by suspending over the fluid a strip of paper moistened with alkaline sugar of lead. A positive test does not suggest the presence in the stomach of any particular organism.

The **gastric sediment** should be studied with care since it often yields evidence of value in diagnosis.

Lyon's[13] method is as follows. He uses a small metal capsule 1.5 cm. long by 6 mm. in diameter with a shaft 4 mm. long by 3 mm. in diameter, to which a small capillary rubber tube is securely fastened by a silk thread. The capsule is perforated at the extreme tip with a hole 1.5 mm. in diameter, in a line continuous with the caliber of the tube, and the body of the capsule is similarly perforated with 8 additional holes 1.5 mm. in diameter. To facilitate cleaning the capsule is made in 2 parts which unite by a screw thread. The capillary rubber tube is 1 meter long and of various calibrations, but should be at least 3 mm. in diameter. This tube can be readily swallowed with minimal discomfort and can be left in situ for several hours to admit of fractional analysis of the gastric juice after an Ewald breakfast, or allowed to pass into the duodenum for the recovery and analysis of duodenal or jejunal contents.

The patient swallows the capsule and tube on an empty stomach, preferable in the early morning fasting state. Then by means of a 1- or 2-ounce aspirating syringe, with a capillary tip and a close-fitting asbestos plunger, gentle aspiration is made to recover the residual contents of the fasting stomach. From 100 to 150 c.c. of plain, warm water is next introduced by means of the syringe and is gently aspirated and then forced back again into the stomach, perhaps a dozen times. The lavage water, which was at first macroscopically clear, soon becomes gradually turbid and contains variously-sized flocculent bodies ranging from pin-point to 3 to 5 mm. in size. After thus douching the gastric mucosa all of the fluid is aspirated from the stomach. A small portion is tested for occult blood and the remainder mixed with equal volume of a 10% solution of formalin. This is then filtered, the filter paper is punctured and the residue washed into a clean bottle with the 10% formalin solution and allowed to stand for at least 3 hours. The residual contents obtained without lavage is treated in the same way.

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[13] Am. Jour. of the Med. Sciences, Sept., 1915, No. 3, vol. cl, p. 402.

The sediment is filtered through a smooth filter paper and the sediment washed down to the tip of the filter by means of a wash bottle. The tip of the filter paper containing the sediment is then cut off and the paper folded so that it encloses the sediment. This package is now wrapped in 1 layer of gauze and tied fast with a thread. This is placed in Acetone I for 1 hour, then in Acetone II for 1 hour and in Acetone III for 2 hours. It is then transferred to paraffin and chloroform, 1 hour, to paraffin (M.P., 52° C), 1 hour, paraffin (M.P., 52° C), 2 hours, the paper then removed and the mass of sediment imbedded in paraffin and serial sections cut and fastened to slides. These are heated in a dry heat sterilizer at 70° to 80° C. for 30 minutes or until the sections are perfectly dry. The slides are then run through xylol (in Coplin's jars) for 5 to 10 minutes, absolute alcohol for 5 minutes, 95% alcohol for 5 minutes, 80% alcohol for 5 minutes, and water for 5 minutes. They are then stained in hematoxylin for 5 minutes, washed with water and then 1% eosin and again washed. They are next run through 80%, then 95%, then absolute alcohol for a few seconds, cleaned in xylol and mounted in balsam.

In sections from the aspirated residue of the normal fasting stomach one finds occasional epithelial cells and occasional leukocytes with protoplasm intact in those cases in which chemical titration shows faintly acid or neutral or slightly alkaline reaction. Boas and Paul Cohnheim have pointed out that digested protoplasm of epithelium cells or leukocytes indicates the presence of free hydrochloric acid and pepsin. When the protoplasm of the epithelial cells is still intact it is possible to differentiate endogenous gastric cells from those originating from the mouth, pharynx, respiratory tract and esophagus. One frequently encounters the snail-like bodies first described by Jaworski which Boas and Paul Cohnheim believe to be mucus acted upon by hydrochloric acid. If there has been regurgitation from the duodenum there may be crystals of some of the bile salts.

Pathologically, in the fasting morning stomach one may find remnants of food eaten the night before, such as muscle fibers still striated or partially digested; starch granules; vegetable-cells; seeds from berries, any of which from a 12-hour fasting stomach is indicative of motor insufficiency due either to pyloric obstruction or rarely to advanced atony, although it should be remembered that small amounts of food remnants are not necessarily pathological (crypts of mucosa and cavities in teeth). Associated with this, if one finds sarcinæ in numbers or many yeast cells in process of germination it would suggest gastric dilatation with stagnation and fermentation. Sarcinæ are rarely found in the ectasis of cancer, except in those cases of the ulcus carcinomatosus type.

Infusoria, Trichomonas hominis and Megastoma entericum may be found. These require for their development an absence of hydrochloric acid, an alkaline medium and a mucosa with crypts. Mucus from the respiratory tract will float, owing to its air content. Microscopically, it is characterized by the alveolar cells and myelin drops which it contains, while columnar epithelium indicates its derivation from the gastric mucous membrane. Also, in gastric dilatation one occasionally encounters spores and mycelial cells from vegetable moulds. Leukocytes, indicative of an inflammatory reaction, are present in large numbers in all cases of gastric ulcer, in many of the simple forms of gastritis during the inflammatory or congestive stage and in cancer of the stomach affecting chiefly the glandularis. Large numbers can be found in some cases of gastric cancer, while in a very few cases of gastric cancer and of subphrenic abscess perforating into the stomach the stomach may contain even from 60 to 500 c.c. of pus. One would expect to find pus in gastritis phlegmonosa and in local abscess of the wall of the stomach. Pathologically, a significant finding is the presence of Opper-Boas bacilli (see page 378). They are large non-motile bacilli with a somewhat typical morphological arrangement in long chains and are readily differentiated from the Leptothrix buccalis by acting negatively to Gram's stain. In gastric sediments prepared as above described they have a tendency to arrange themselves in dense masses, interlaced with one another and resemble hair-like balls when viewed under a low-power microscope.

The normal stomach should contain very few bacteria. When the bacterial flora is abundant it indicates a distinctly pathological condition. The most common normal invaders of the stomach is the Bacillus coli group, but the appearance of diphtheroid bacilli, staphylococci and particularly various types of streptococci indicates local infection. Here, too, one meets with a pathologically increased number of leukocytes.

It is surprising how often small isolated fragments or flakes of gastric mucosa will be recovered by this method. Minute particles, barely of macroscopic size, which would readily escape detection in the lavage water, may prove to be the one point upon which the correct diagnosis can be made. It is often possible from a microscopic study of these bits of mucosa to determine from which segment of the stomach they come, whether the fundus, prepylorus, or antrum pylori, bearing in mind the anatomical distribution of the different types of glands. Microscopically, these minute fragments may show only the peripheral portion of the villus extending down to various depths through the glandularis, while in the larger fragments the entire width of the mucosa, at times including the muscularis mucosæ, will be found. Some are clearly fragments of cancer. Fragments of normal gastric mucosa are not infrequently found in the stomach contents by those who attach a suction pump to the stomach-tube when emptying this organ. These men claim that the injury to the gastric mucosa which such strong negative pressure produces is of no importance, an opinion which needs confirmation. Others, more gentle with the mucosa in their technic, learn much from the bits of mucous membrane whose presence indicates that vulnerability present in malignant disease, in chronic gastritis and in some cases of Heynoch's purpura with edema of the stomach wall (Morris). The fragments of proliferating mucosa thus obtained in some cases of achylia gastrica closely resemble bits of carcinomatous tissue. Fragments of tumor should be searched for in the morning stomach washings of patients suspected to have gastric cancer. In these washings the fragments of tumors, the chains of long bacilli and the parasites' eggs are the most important finds.

Microscopic Examination.—The microscopic inspection of the partially digested food of a test meal seldom yields any results of value, although mucus and pus can best be recognized in this way. Nothing can be learned from the inspection of the muscle fibers.

Infusoria (see page 362) are sometimes found in the gastric contents of patients whose gastric juice has for some time been neutral or alkaline in reaction.

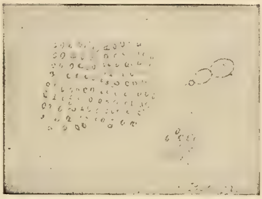

Fig. 71.—Sarcina ventriculi and yeast cells. × 900.

In case of gastric stasis with free HCl present, *moulds*, *yeasts* and *sarcinæ* will predominate; if there is no free HCl, bacteria. Einhorn found in the wash-water of certain cases the spores of moulds which he thought lodged in crevices of the mucosa and might produce the pyrosis and gastralgia present. A few yeasts (Fig. 71) may be found in normal stomachs but they are abundant in cases of gastric dilatation. Sarcinæ (Fig. 71) occur in large numbers in stomachs with benign dilatation, occasionally in gastritis, ulcer, and gastric neuroses, but rarely in cancer. One observer reported them in such numbers as to form plugs obstructing the pylorus. Ehret found many sarcinæ in cases with intense fermentation of the gastric contents, but no yeasts nor bacteria. He considers that their presence indicates a marked stasis. Two sizes may be found, the large and the small. In a recent case with dilated stomach in this clinic, sarcinæ, huge in size, were present in great numbers.

Blood.—Traces of fresh blood are often present in the gastric contents. It may come from the esophagus, nose, mouth or lung, but more commonly its presence is explained by the slight lesions of the pharynx, esophagus or stomach produced in vomiting or by the tube. Blood in large amounts is found in cases of ulcer, some cancers and from rupture of venous dilatations at the cardiac orifice, as in portal obstruction. If the stomach was empty at the time of the hemorrhage the blood may appear arterial, but as a rule it is dark because of the hematin produced by the gastric juice, is clotted and is well mixed with food.

Definite gastric hemorrhages occur also in chronic passive congestion; in cirrhosis of the liver; in various constitutional diseases in which the reason for the bleeding is not apparent, as in the anemias, hemophilia and Hodgkin's disease; in active hyperemia of the gastric mucosa, as in vicarious menstruation; and following abdominal operations, especially such as involve the omentum, in which case they are a disturbing symptom but are of no moment. All the blood of even a profuse gastric hemorrhage may pass by the stools.

In carcinoma of the stomach, in which a slight constant oozing from the tumor is the rule, the blood is well mixed with the food, is digested and looks like coffee grounds. This in a case of gastric dilatation always suggests cancer and yet may be present also in cases with hyperacidity and hemorrhagic erosions of the mucosa. Such blood must be recognized chemically. Blood may arise also from tuberculous ulcers; from slight injuries to the mucosa; from small aneurisms of the gastric arteries; and from infarcted areas.

Occult hemorrhages, that is, hemorrhages so slight that the presence of blood would not be suspected by inspection but must be detected chemically, are common in several conditions which Boas and Kochmann[14] classify as follows: Those in which the blood is never evident, as gastritis anacida, subacidity, hypersecretion, benign ectasis; cases with at times gross hemorrhages, especially ulcer; cases with evident blood as a rule, cancer especially.

Deen's Test for Occult Blood.—To the gastric juice are added 1 c.c. of fresh tincture of guaiac and 1 c.c. of Hühnerfeld's solution (glacial acetic acid, 2; distilled water, 1; oil of turpentine and alcohol, of each 100 c.c.). The mixture is well shaken. If blood is present the fluid will turn blue. The test is also given if iron compounds and some vegetables are present, hence it had chiefly a negative value. *Weber's modification* is recommended by Riegel. To the gastric contents is added ⅓ its volume of glacial acetic acid and it is shaken out with ether. After the ether extract has cleared, to a few cubic centimeters are added 10 drops of guaiac tincture and 20 to 30 drops of turpentine. Blood will give a bluish-violet color. In this case only raw or rare-cooked meat are to be excluded.

For the *spectroscopic test*, the gastric contents are diluted with water, a few drops of concentrated acetic acid added and it is shaken out with ⅕ its volume of ether. In a few minutes a clear layer of the brown ether solution of hematin will be obtained. Since the 4-band spectrum of hematin in acetic acid could be due also to chlorophyll, an alcoholic solution of KOH is added and the hematin reduced with $(NH_4)_2S$. The resulting red fluid will give the 2-line of reduced hematin.

Motility of the Stomach.—Any disturbance of the motility of the stomach is of far more importance than are disturbances of secretion. If the motility is good the person may live for years in ignorance of the fact that he has no gastric juice. But if motility be impaired the symptoms will be severe and the stagnation of food in the dilated stomach will soon produce serious results. It is of interest that the symptoms of hyperacidity (heart-burn, acid eructations, the vomiting of acid fluid, etc.) are due to disturbed motility and bear little direct relationship to the actual degree of gastric acidity.

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[14] Arch. f. Verdauungsk., 1902, vol. viii, Heft. 1, 2.

The most rapid motility is seen in cases of jejunal fistula high up. In these cases of starvation the stomach seems to hurry the food into the intestine at as rapid a rate as possible.

It is quite important in all diagnostic work to wash the stomach well in order to be sure that it is empty, since in cases of achylia the tube may siphon nothing and yet the wash-water show considerable solid matter.

Megalogastria means enlargement of the stomach. This may or may not be accompanied by motor insufficiency.

Ectasis refers to enlargement with motor insufficiency. The term " atony " is used if it is due to real weakness of the muscle wall; " hypertonic ectasis " when due to pyloric stenosis.

MOTOR INSUFFICIENCY of the stomach may be absolute or relative and the resulting dilatation is in general due to 1 of 2 factors: (1) To atony of the gastric wall, in which case the muscle is not strong enough to empty the stomach; in this group are found the largest stomachs. Strauss [15] reported 1 with a capacity of 5½ liters. (2) Relative muscular insufficiency. By this is meant that while the muscularis may be abnormally strong and hypertrophied an obstruction at the pylorus renders the exit of food difficult. As long as the hypertrophy of the muscle can compensate for the obstruction there will be no dilatation of the stomach.

A *pyloric stenosis* may be congenital or acquired. Acquired stenosis may be due: to the contraction of scars of ulcers; to cancers; to hypertrophic stenosis (said to be the result of a continued pyloric spasm stimulated by hyperacid gastric juice or by an ulcer) or to scars resulting from irritating poisons. Or, the obstruction may be due to the pressure of neighboring tumors; to twists; to diverticula; and, finally, to malpositions of the pylorus due to adhesions.

Disturbances of gastric motility explain most of the gastric symptoms usually ascribed to disturbed secretion, etc. Undoubtedly the best method of determining the efficiency of the gastric motor mechanism is the röntgenological observation of the time necessary for this organ to empty itself entirely of the buttermilk-bismuth subnitrate or barium sulphate meal. The stomach should be entirely clear of such contents in 6 hours.

In case of food the normal stomach should be empty in 7 hours no matter how large the meal. A common test of motility and a method of estimating the degree of insufficiency is as follows (Boas): The patient, whose stomach has not been washed out, is given a simple evening meal but one of constant composition, as cold meat, bread and butter and tea. On the following morning the stomach is thoroughly washed out. If food is found the motor insufficiency is marked (Grade B). On the following afternoon the stomach is well lavaged, the same meal is repeated and on the next morning again washed. If the stomach is found empty the insufficiency is less than B (Grade C). If food is found it is more than B (Grade A). If the stomach contains food 7 hours after a full noon or morning meal, but none after a night's rest, the degree of insufficiency is least (Grade D).

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[15] Deutsch. med. Wochenshr., 1904, No. 15.

Ewald and Strauss have recommended that 1 spoonful of currant or raisin preserve be given with the evening meal. These seeds can be recognized the next morning in the stomach washings even though the patient has taken a large breakfast.

If before breakfast the stomach contains over 100 c.c. of fluid motor insufficiency may be suspected and lavage will probably wash out food particles, but such a stomach will contain no fluid if it is washed out the evening before.

The symptoms of extreme dilatation are those of the primary disease together with the vomiting of food eaten more than 7 hours, in some cases even 3 days, before. The repeated vomiting of food in the morning before breakfast is conclusive proof. But the symptoms of less marked disturbances of motor function are not as characteristic. The most constant of these is a discomfort in the epigastrium which begins about 3 hours after a meal when the expulsive movements begin, and which is relieved by soda or by a lunch. "Heart-burn" is evidence of slight retroperistalsis, the eructations of acid fluid of a slightly more marked grade, and nausea and vomiting of a still more severe retroperistalsis.

For those patients who object strenuously to a stomach-tube and who have not access to a röntgenological institute, various chemical tests have been proposed. That of Ewald and Sievers is based on the belief that *salol* remains unchanged in the stomach but is split by the pancreatic juice and by bacteria to salicylic acid and phenol. The salicylic acid may easily be detected by the violet color of the urine when ferric chloride is added. This test assumes that the time of splitting and of the absorption of the salol and the time of excretion of the salicyluric acid remain constant, which is not quite true. One gramme of salol is given with the test breakfast and the urine is examined at intervals. The acid should appear in the urine at the latest in 75 minutes. But since the salol may enter the duodenum with the first portions of food as well as with the last and since, in cases of achylia, bacteria can split some of the salol (and mucus can also) Huber recommends that we test not alone the time of the appearance but also of the disappearance of the salicyluric acid, which should be in from 26 to 27 hours. The urine is therefore examined 27 hours after the meal, and, if found, repeatedly at intervals of 3 hours.

Sörensen and Brandenburg [16] recommend that we give the patient when the stomach is empty 300 to 500 c.c. of 3% protogen. One removes as much of this as possible in from ½ to 1 hour. From 100 to 200 c.c. of water are then introduced and as much more as possible removed. The nitrogen in both fractions is determined by the Kjeldahl method and from this the amount in the stomach calculated.

The composition of the gastric juice in dilated stomachs will depend on the disease causing the dilatation. In general there are 2 groups of cases, one with acid and the other with anacid contents. The former includes cases of ulcer, the latter those of cancer and chronic gastritis. As a rule the mucosa of a dilated stomach becomes less sensitive to stimuli, owing perhaps to the constant presence of food and to the gradual development of a chronic gastritis, and so secretes less and less acid.

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[16] Arch. f. Verdauungskrankheiten, Bd. 2.

Of 45 of our benign cases, 7 were hyperacid, 15 showed normal acidity and 9 were hypoacidity with, and 4 without, any free hydrochloric acid.

By " hyperacidity " is meant the secretion of abnormally acid gastric juice during digestion, that is, while there is a normal stimulus for secretion. By " hypersecretion " is meant the secretion of an amount of gastric juice out of proportion to or in the absence of a physiological stimulus. With the exception of a few cases of nervous disease it is doubtful whether either occurs often enough to be important.

Hyperacidity, Superacidity, Hyperchlorhydria, or Hyperaciditas Hydrochlorica.—The secretion of a very acid gastric juice, that is, one containing an abnormal per cent. of free hydrochloric acid, has been assumed as the explanation of many gastric conditions with pyrosis, etc. In recent years the demonstration that gastric juice is normally much more acid than was supposed, that extreme acidity need give rise to no symptoms, that the heart-burn, acid eructations, the vomiting of acid gastric juice, etc., are due usually to pyloric spasm and to slight motor insufficiency, and, finally, that the chemical analysis of the fluids which symptoms would indicate to be very acid usually are not, have thrown considerable doubt on the importance of hyperacidity as such (see page 349).

Hyperacidity formerly was attributed to diseases of the central nervous system, to a defective nervous control of secretion and to changes in the mucosa. This term was used of cases with an acidity per cent. over 70. Often it is 100, sometimes it is 150 to 160 or more and the free HCl from 60 to 80. Following the test meal the total is over 70, even 100 or more, and the free from 50 to 60. Others (Meunier) say that the acidity alone is not important, but that the specific gravity of the contents must be low, from 1.007 to 1.019 instead of from 1.022 to 1.040, as normally.

The majority of the cases formerly termed hyperchlorhydria are doubtless cases of duodenal ulcer, of gall-stones or of subacute appendicitis. Nevertheless some are secretory neuroses. For this last diagnosis the acidity should be very variable and vary definitely with the nervous symptoms, an important point in a diagnosis at best difficult.

Hypersecretion, Supersecretion, Continuous Secretion, "Gastrosuccorrhea."—In cases of continuous secretion the formation of gastric juice is supposed to continue when the stomach contains no food, which would suggest as cause a disproportion between stimulus and response. The free acidity in such cases is relatively high, which is a valuable point in ruling out cases of motor insufficiency. Continuous secretion is proven by finding much acid gastric juice in a previously well washed fasting stomach. This condition may be constant or intermittent. It may also be reflex as from a duodenal ulcer; a part of a general neurosis; a secretory neurosis, or the result of organic nervous disease. Among the last may be mentioned the gastric crises of tabes dorsalis. Among the more constant cases are those of gastroxynsis (Rossbach) and those seen in neurasthenia and hysteria, in myelitis, in general paralysis and even after the excessive use of tobacco. In the periodic or intermittent cases of *Reichmann's disease*, the patient's digestion during the intervals may be perfectly normal, then there occur sudden pains, acid eructations and the vomiting of a cloudy yellowish fluid, which at first contains food, then is pure gastric juice measuring often several hundred cubic centimeters in amount, the acidity of which may be normal or increased (the latter usually only when food is present).

The chief symptoms of the continuous cases are a feeling of discomfort and of weight in the epigastrium, eructations of acid fluid and pain which begins about an hour after eating and increases until the stomach has emptied itself into the duodenum or until the patient has vomited. Vomiting about midnight is a very characteristic symptom. The patient usually vomits large amounts (from 500 c.c. to 1000 c.c. or much more) of a thin fluid the acidity of which is normal or slightly above the normal. There is often pain before meals, which is relieved by eating.

Many doubt whether continuous hypersecretion is ever " functional." They claim that it is always caused by some gross stimulus to secretion, as ulcer, stenosis of the pylorus, or by some condition favoring the retention of particles of food.[17] Foster and Lambert[18] have shown that pyloric stenosis is a sufficient cause of continuous secretion; that the presence of retained food particles is not necessary to explain it. Other cases called nervous are supposed to be due to reflex disturbances from the intestine and are relieved by treating this organ.[19]

To diagnosticate chronic hypersecretion, Riegel recommends the following routine. The stomach is emptied after a full meal at the height of digestion and its contents measured. The next step is to pass the tube some morning after a night during which the patient has eaten and drunk nothing and note the quantity of clear fluid obtained. Over 100 c.c. is considered pathological. Lastly, at evening the stomach is washed and finally emptied (very carefully, since it is hard to wash out all the food) and the contents obtained the following morning noted.

A case reported by Thayer will serve as a good illustration. The symptoms were of 2 years' duration; the total acidity after the Ewald breakfast was 113; the fasting stomach always contained even 420 c.c. of acid fluid, the acidity per cent. of which often was 117. Digestion was good.

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[17] Kaufmann, Am. Jour. Med. Sci., 1904, vol. cxxvii.
[18] Jour. of Exp. Med., 1908, vol. x., p. 820.
[19] Faber, Arch. f. Verdauungskrankheiten, Bd. 7.

Nervous Dyspepsia.—Hyperacidity, hypersecretion, anacidity, etc., acid eructations, gas and sensations of all descriptions, some very painful, are symptoms of conditions which may be due to organic changes of the mucosa, to functional disturbances following bad habits of eating, poor food, etc., to reflex stimulation from gall-bladder or appendix or be a part of a general neurosis, the "nervous dyspepsia" so common in this country that the stomach specialists abroad speak of it as the "American disease." It is exceedingly difficult to separate the element due to food, rapid eating, disease, etc., from the reflex and the neurotic elements and in the majority of cases perhaps 2 or 3 coexist. The nervous dyspeptic usually has some organic reason for his gastric distress although a neurasthenic will often worry his normal subliminal gastric sensations into the sphere of consciousness. He is nervous and he has gastric symptoms. It is, however, a great mistake to consider the latter entirely a nervous trouble for the majority have a lesion which demands treatment. Among these are inflammatory conditions of the nose and nasal sinuses, tonsillitis, pyorrhea alveolaris, stomatitis, chronic glossitis, etc. Considering the amount of pus which these patients swallow we may well admire the resistance of the stomach. Among other causes are ulcer of stomach or duodenum and disease of the gall-bladder or appendix.

Some of these "neurasthenics," have hyperacidity; more, slight subacidity; and many, normal acidity. Their subjective symptoms bear very little relation to the condition of the gastric juice. A patient with normal gastric juice or with hyperacidity may describe sensations quite similar to those of an anacid case, except perhaps that the hyperacid case is more apt to vomit than either of the other two.

In the Johns Hopkins clinic we studied the records of 300 such cases admitted during 4 years. In 82 there was hyperacidity and in 20 others the clinical features were those of hyperacidity although the total acidity was not over 70. Subacidity (total acidity less than 40) was present in 170 cases, in 61 of whom there was no free hydrochloric acid, and in 4 of these the fluid was practically neutral to litmus.

In conclusion, we would urge that the diagnosis "nervous dyspepsia" be made only of cases definitely neurotic whose gastric function is usually quite normal and whose periods of dyspepsia would seem directly related to neurotic states. The great majority of cases of so-called nervous dyspepsia are true dyspepsias of nervous persons who have definite organic basis for some at least of their troubles.

Acute Gastritis.—By acute gastritis is meant an acute irritation or definite inflammation of the gastric mucosa, resulting, in mild cases, in increased mucus secretion and in more severe cases in an acute inflammatory reaction with desquamation of the epithelium, and in all with some disturbance of secretion. It may be due primarily to the direct irritation of unsuitable foods, to poisons of all sorts, or to heat, cold, etc.; or, it may be a part of an acute infectious disease. The vomitus of these cases is acid, rarely neutral, in reaction, has a bad odor, is often fermented, the food is undigested as a rule and mixed with much mucus. The total acidity is diminished, free hydrochloric acid is absent as a rule and organic acid is often present. If there has been much retching the vomitus contains more or less bile. A test meal will show mucus, undigested food and little or no free HCl.

We have records of but 5 clear cases, all of whom had subacid or neutral gastric contents.

GASTRITIS PHLEGMONOSA or INTERSTITIAL PURULENT GASTRITIS is a very rare condition with inflammation of the entire gastric wall even to the serosa. When localized a gastric abscess is the result. Vomiting is a common symptom. In the 60 diffuse cases reported pus was not found in the vomitus (Riegel). It was found in a very few reported cases of abscess of the stomach wall.

In the *gastritis acuta purulentia* (Leube) the inflammation is limited to the mucosa.

Chronic Gastritis.—Chronic gastritis is not nearly as common a condition as one would imagine from the number of times this diagnosis is made. The term implies a definite long-standing inflammation of the mucosa which leads to definite atrophic changes of this membrane and to weakening of the muscularis. It exists in all grades even to complete atrophy of the mucosa. Among the common symptoms are a great increase of mucus in the stomach washings; vomiting, especially on an empty stomach in the morning or at the height of digestion, of undigested or poorly digested food mixed with mucus; and motor insufficiency. These cases have been classified as primary, *i.e.*, without demonstrable disease as adequate cause (although more and more the chronic pyogenic infections of the nose, throat, tonsils and teeth are considered satisfactory explanation) and secondary, or accompanying other diseases as ulcer or cancer of the stomach or pyloric stenosis from any cause whatever.

The amount of gastric contents removed after a test meal is about normal. The food has the appearance as if it were just swallowed, except that it is intimately mixed with much mucus which renders its removal through the tube difficult and its filtration tedious. The amount of mucus present is best estimated by macroscopic examination. The stomach must be washed thoroughly and repeatedly since the most of the mucus appears in the later washings.

In chronic gastritis the secretion of gastric juice diminishes progressively as the case advances until the achylia may be complete. The total acidity varies much and at times some of the acid may be free, hence a correct diagnosis requires repeated examinations. The secretion of hydrochloric acid decreases first; that of the ferments second (Bouveret believes that the easiest way to follow the progress of a case is to observe the rennin secretion); that of water next; while during all this time the secretion of mucus may even increase. Proteid digestion is much impaired, that of carbohydrates not at all. The stomach often becomes somewhat (seldom much) dilated since its walls are weakened. The pylorus may be slightly obstructed by inflammatory thickening of its mucosa and slight motor impairment result allowing fermentation, yet seldom will more than a trace of organic acid be found in the contents. The presence of much undigested food in the stomach washings does not necessarily indicate stasis since it is a function of the stomach to retain food until it is digested. In many cases, however, the gastric motility is normal or even increased and symptoms of stomach trouble absent since the intestine vicariously will fulfill the gastric function. In cases of chronic gastritis the mucosa of the stomach is abnormally fragile and one often finds fragments of mucous membrane in the stomach washings.

Some cases begin with an actual increase of the gastric acidity. The fasting stomach of these cases contains mucus enclosing many cell nuclei and an hyperacid juice. The reason these cases of gastritis acida would seem to be rare may be that this stage has passed before the patient consults a physician.

Of 27 of the Johns Hopkins Hospital cases, 1 was slightly hyperacid (72 total acidity), in 10 the acidity was within normal limits and in 15 it was below 40. Nine of these 40 had no free acid. Four could be termed atrophic catarrh. In 1 case only 1 c.c. of bile-stained mucus could be obtained by the stomach-tube and at autopsy was found cirrhosis of the stomach wall.

Mucus.—A little mucus is usually demonstrable in the contents of normal stomachs especially towards the end of the digestion period. Mucus is present in excess under the following circumstances: if the diet is particularly rich in starch; in conditions of subacidity and anacidity of the gastric juice, though in these cases the increase is sometimes only apparent, for, while the normal amount may have been secreted an amount less than normal may have been digested; if the stomach contents are irritating to the mucosa, which seems to protect itself in this way; rarely, in cases of gastric hyperacidity, and these all may belong in the preceding group; and finally in all forms of chronic gastritis (" gastric catarrh "), both the primary form and that which accompanies cancer of the stomach, pyloric stenosis, etc. The largest amounts of mucus are found in cases of developing achylia. It would appear that the secretion of mucus increases as that of the gastric juice decreases. A correct idea of the amount of mucus present in the stomach can be gained only by repeatedly filling and emptying the stomach, or, better still, by the use of a needle douche-tube, since it sticks tenaciously to the gastric wall.

Mucus from the stomach is present as delicate transparent flakes, which are mixed with the food, which sink in water, and which contain the nuclei of leucocytes. Mucus from the air passages is present in glassy balls, which float (they enclose air), which contain cylindrical epithelial cells and often visible pigment and which are not mixed with the food. Foster [20] attributes to the mucus the diminution of, and the variations in, the amount of free HCl in cases with a high free acidity, for the products of the digestion of mucus have a high acid-binding power.

Atrophy of the Mucosa—Achylia Gastrica.—Achylia gastrica may be due to a functional disturbance of an apparently normal mucosa or to real atrophy of this membrane. The latter may be the end stage of a chronic gastritis or accompany cancer and other diseases which lead to degenerative changes of this mucosa. One must remember that the diagnosis of achylia very often is an error since the meal was not removed at the proper time (see page 354).

Some cases of achylia are of nervous nature, and the discovery purely accidental. In other cases the nervous symptoms may disappear but the achylia continue. When due to atrophy the process is gradual and the secretion may diminish until finally there is almost no gastric juice. There is an interesting group of cases of achylia associated with very small gastric cancers which is apparently toxic in character since much of the mucosa looks normal. Achylia gastrica sometimes accompanies cancer in other organs (breast, intestine, esophagus, uterus, etc.) and is present before any disturbance of the general health is manifest and may be due to other conditions which lead to general malnutrition. The cases of particular interest are those which simulate pernicious anemia and those with protozoon infection of the bowels.

A severe achylia may not be suspected provided the motility of the stomach is good (the intestine seems to act vicariously) although if even slight motor insufficiency has developed the symptoms will be evident enough.

An atrophied mucosa is very susceptible to injury. It is indeed not uncommon to find in the stomach washings pieces of diseased mucosa. Such cases often vomit undigested food soon after eating, which seldom contains blood. The diagnosis of achylia due to atrophy is made with the Rehfuss stomach-tube, testing the gastric contents at frequent intervals until the stomach is empty. The food removed is little changed. The total acidity is very low, from 1 to 4, there is no free HCl and lactic acid is rarely present except in cases of severe ectasis. The ferments may be absent, an important point in diagnosis. In most cases the washings with the larger tube contain great quantities of mucus for the mucosa may consist chiefly of mucus cells; the glandular cells have practically disappeared. In extreme cases even mucus is absent. To obtain nothing through the tube by simple siphon action does not necessarily mean that the stomach is empty and clean, since subsequent lavage may remove dry food. All of these findings should be confirmed by several test meals.

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[20] Am. J. of Med. Sci., Feb., 1907, vol. cxxxiii; and Med. Record, Aug. 13, 1910.

Ulcer of the Stomach and Duodenum.—In general the clinical picture is the same whether the ulcer is on the gastric or duodenal side of the pylorus. The clinical types of this disease are: (1) The latent ulcer which may pass unsuspected unless hemorrhage or perforation occurs. This is seldom near the pylorus or in the duodenum.

(2) The hemorrhagic form, which may be acute and sometimes fatal, or chronic, the frequent small hemorrhages causing considerable anemia and cachexia. The stools of these patients constantly contain a certain amount of blood.

(3) The acute perforative type.

(4) The chronic dyspeptic type in which the symptoms of "indigestion" may persist for years. These ulcers are usually near the pyloric ring.

(5) The neurotic, or gastralgic type.

(6) The vomitive form, with vomiting the worst symptom.

(7) The cachectic form which presents the picture of a cancer.

The cardinal text-book symptoms of gastric ulcer are: (1) Gastric symptoms usually of long duration and beginning as a rule in young adult life. (2) Pain, paroxysmal and local, from half an hour to 2 hours after the meal, *i.e.*, when peristalsis is most active. (3) Vomiting of acid vomitus containing well-digested food usually 1 to 3 hours after the meal but also often in the morning, and followed at once by a diminution of the pain. Hemorrhage, in from 30 to 50% of the cases and in these only occasionally. This blood as a rule is dark in color (due to the hematin formed by the hydrochloric acid) although if the stomach is empty at the time of the hemorrhage it may be arterial. There is often occult blood in the stools but it is not present as constantly as in cancer. If the blood remained for some time in the stomach before it was vomited or removed through a tube it may resemble coffee grounds. In such cases iron, wine, coffee, medicines and certain foods must be excluded. Other reasons for hemorrhage must be excluded, as tuberculosis, cancer, chronic passive congestion, cirrhosis of the liver, rupture of an esophageal varix, etc. (5) Hyperacidity is a classical symptom but the subjective sensations of this (pyrosis, acid eructations, pain, etc.) often do not coincide with the chemical findings and are due more to slight retroperistalsis than to hyperacidity. Ewald, using the Riegel meal, found in 75 cases that the total acidity averaged 105 and the free HCl 50, with a maximum of 89. But these figures are not very high and in at least half the cases the acidity is diminished. Many separate 2 groups, the "fresh" and the "old" ulcers, and find that in the latter the acidity is much lower. Repeated examinations should be made in order to get a correct idea of the acidity conditions.

In general in ulcer cases digestion is good and motility is rapid.

The results of a gastric ulcer may be stricture of the pylorus or severe anemia due to the insufficient nutrition, vomiting and hemorrhages. In case the blood is lost chiefly by the stools, as in duodenal cases, the ulcer may be unsuspected and the case be diagnosed pernicious anemia. It is said that in case a cancer develops on the bed of an ulcer the acidity may for a long time not be lessened, but in most cases it gradually diminishes.

The 82 cases of the Johns Hopkins Hospital medical clinic were reported by Howard.[21] Vomiting was present in 85.3%, especially in the cases with ulcer at the pylorus. Blood was vomited in 75.6% of the cases although in ⅓ only was the blood bright red. After the test breakfast more than 50 c.c. was obtained in 54% of the cases. Hyperacidity was present in 27.5%, in 42.5% subacidity (for these figures an acidity per cent. of 60 was considered the upper limit of normal). In 18% there was no free HCl, while in 14% lactic acid was present.

The laboratory tests of duodenal ulcers are as unsatisfactory as the clinical evidence is easy. Hunger pains entirely relieved by eating, "heart-burn," acid eructations or vomiting from 1 to 5 hours after a meal relieved by further eating or by soda, together with the intermittent presence of blood in the stools are enough for diagnosis.

HEMORRHAGIC EROSIONS.—It is doubtful whether there is any symptom complex of hemorrhagic gastric erosions which is characteristic. The most suggestive evidence would be fragments of mucosa without marked pathological changes and found in the washings of the empty stomach. Vomiting is rare. The acidity in such cases is normal or diminished, rarely increased.

Syphilis of the Stomach.—Lyon [22] writes that the diagnosis of syphilis of the stomach is justified if the serological examinations are definitely positive and if syphilitic therapy results not only in a general clinical improvement but also in a cessation of gastric symptoms.

This is hardly true since gastric symptoms may be evidence of disturbances not in the stomach but in other organs, as the gall-bladder, appendix, etc., and in luetic patients these may be relieved by antisyphilitic treatment, thus relieving the gastric symptoms.

Being a tertiary lesion gastric lues is far more apt to become manifest during the middle decades of life, it may affect both sexes and may be a result of both congenital and acquired infection.

Pathologically the disease may show any one of the following forms: (1) A diffuse gastritis; (2) syphilitic ulcers; (3) a diffuse infiltration of the gastric wall; (4) pyloric stenosis and (5) gumma, which may or may not give rise to a palpable tumor.

The secretory defect is usually accompanied by the symptoms of a severe atrophic or sclerosing gastritis.

The gastric analysis shows a marked subacidity or anacidity with a greatly diminished or absent enzyme activity. On the other hand a few cases have been reported in which the hydrochloric acid content and peptic activity were normal or even increased. An increase of endogenous mucus is generally the rule. Occult bleeding is frequently encountered both in the gastric filtrate and in the feces.

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[21] Am. Jour. Med. Sci., December, 1904.

[22] The Arch. of Diag., Apr., 1917.

Cancer of the Stomach.—Clinically cases of cancer of the stomach may be separated into 3 groups: the latent; those with cachexia but no gastric symptoms; and those with definitely localizing symptoms. The important points in the diagnosis of a quite advanced and typical case of this disease are: a history of the rather sudden appearance of dyspeptic symptoms in a person beyond 40 years of age, which symptoms were new to that person; loss of weight and strength; anemia; the lack of free hydrochloric acid and the presence of lactic acid in the gastric contents. But " typical " cases are rare. The sudden and apparently inexplicable loss of weight and strength and the development of a secondary anemia in an adult should always arouse our suspicion of malignant disease and yet such a case could not be called " early." It probably would be far advanced but so situated that its presence as a tumor could give no localized symptoms. It might be anywhere in the body. The additional symptoms which would indicate that the cancer involves the stomach may be divided into 2 groups: Those depending on the fact that the stomach is the organ involved and those which depend on the location of the cancer in the stomach wall.

Undoubtedly the earliest and most important symptoms of gastric cancer are: a change in the appetite, especially a loss of appetite for meat, slight dyspeptic symptoms such as gas, a heavy feeling after eating, indefinite discomforts, etc., which are new to that patient and which have not been present for more than 2 years. Among the localizing physical signs are: a palpable mass and stenosis at cardiac orifice or at the pylorus. These differ in no way from those due to benign conditions.

Among the early clinical laboratory tests are the almost continuous presence of occult blood in the stomach washings and in the stools and the irregularity in the curve of the free HCl.

At present the röntgenological examination of the stomach is over-popular as a means of early diagnosis of this disease, one reason for which is, we firmly believe, that doctors have not been careful in taking case histories or accurate in their clinical laboratory work. The latter 2 methods are, we are confident, of far greater value in early diagnosis than is generally believed.

Of the later local symptoms and signs, any one of which may be absent, are: vomiting, hemorrhage, subacidity and fermentation. Vomiting is very common (in 85.3% of Osler's first 150 cases), but it depends much on the position of the cancer. If it is at the pylorus causing stenosis the vomiting will be late after a meal but if at the cardiac orifice there will be regurgitation at once after eating. There is least vomiting when the cancer is on the lesser curvature. If there is no stenosis at either orifice there often is no vomiting at all, but it is also true that in 6 of 30 cases with the cancer actually at an orifice there were none. In late cases with dilated stomachs the patients may vomit from ½ to 1 liter or more and in this may be recognized food that was eaten days (in 1 case four weeks [23]) before. The proteins of the food will be found poorly digested, the meat in lumps often covered with mucus, sometimes decomposed and often mixed with digested blood. In other cases the motility is excellent or even increased, as in 11 of 76 cases in which it was hard to get any fluid at all at the end of an hour.

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[23] Osler and McCrae, Modern Medicine.

Some hemorrhage is almost constant in gastric cancer. It is parenchymatous as a rule and yet early it may be profuse and even fatal. As a rule, however, the patient knows nothing of it unless his stomach be washed out or his stools are carefully examined, since it is gradual, and, since the blood is digested, it resembles coffee-grounds and is mixed with food. Of the 150 cases reported by Osler and McCrae, the vomiting of blood occurred but in 21.8%, but chemical examination of the stools shows it present in almost 100% of the cases.

Other symptoms, *e.g.*, the gradual diminution in the amount of secretion, are due to the chronic degenerative changes of the mucosa which begin early and develop late. The absence of free HCl is an early and important sign of cancer, present in over 80% of all cases on first examination, although there is one group without previous symptoms of ulcer which begin with hyperacidity (Zeigler). In 163 cases of Osler's clinic the free acid was absent in 146, or 89%. Alone, a subacidity is not of great importance since in cases of pernicious anemia, of chronic gastritis and of cholecystitis the free HCl may fail, but in cancer one finds a reduction of the free HCl when the total acidity is not diminished and while the total chlorides are high. The free and total acidities vary considerably from day to day. Even in the presence of free acid this may suggest carcinoma and in a recent early case was the point which led to operation. In this case the 3 Ewald breakfasts given during 14 days had the following acidities: total 121, 100, 37; and free HCl, 11, 16 and 10 respectively. In early cases the free acid may suddenly disappear and then following the excision of the cancer may return in a day or two. In cases of carcinoma of the duodenum or esophagus the disappearance of free hydrochloric acid can best be explained as due to a fluid from the tumor which flows into the stomach.

The diminution or disappearance of free HCl certainly is due at first to the binding of this acid by some body which itself does not react alkaline to litmus. The idea originally suggested by v. Velden that the cancer furnishes a secretion which binds the acid is now again advanced. What these bodies are which bind the free hydrochloric acid has been the subject of considerable investigation. Certainly the hydrochloric acid introduced into the stomach of a carcinoma case is soon neutralized (Stähelin). They could be products of the digestion of proteins, albumoses, peptones and hexone bases. Reissner explained the disappearance of free acid in the presence of an undiminished or even increased total acidity on the theory that free alkalies are secreted by the tumor tissue. But if this were the case the chlorides thus formed would be neutral and the total acidity would be reduced. Work which we have done leads us to believe that the tumor furnishes the gastric contents with a proteolytic ferment the action of which produces an abundance of digestion products which can bind the acid and yet allow it thus combined to react as acid to litmus. Fisher [24] has confirmed this work by isolating from the contents of carcinomatous stomachs tyrosin, leucin, arginin and lysin.

Later in the progress of the malignant disease there is a gradual reduction in the amount of total HCl secreted which continues until finally the gastric contents may react as alkaline to litmus. This is due to degenerative changes in the gastric mucosa. On the other hand the secretion of hydrochloric acid may for a time increase as the result of regular lavage, proper dieting and general building-up treatment. In cases of cancer developing on the base of an ulcer the presence of free HCl may continue for a considerable period. In such cases there are often marked fluctuations in the quantity of acid.

Of 64 of our cases with no free HCl the gastric contents after the Ewald breakfast was almost or quite neutral in 8, below 10 (acidity per cent.) in 20, between 10 and 20 in 15, between 20 and 50 in 14 and between 60 and 103 in 7. The higher acidities depended on the lactic (and butyric) acid present.

Later the pepsin and rennin are diminished. This is due to the chronic gastritis and is not specific for cancer.

The presence of LACTIC ACID in the gastric contents is often an early and very valuable sign of cancer. It may be demonstrated in about 90% of all cases when none of the HCl is free although the bound acid may be abundant. It is true that lactic acid cannot be demonstrated in all cases of cancer and also that it may be found in some benign conditions, as in cases with atonic and anacid stomachs and in atrophic catarrh with stenosis of the pylorus. But in the benign cases it is usually absent, even though the stenosis is extreme and the fluid anacid, so that its early appearance in cancer before the stenosis is marked and the total hydrochloric acid much diminished must indicate for it a different significance and emphasizes the value given it by Boas in the early diagnosis of malignant disease, although the specificity he claimed is no longer granted.

Of 609 benign gastric cases lactic acid was present in 30. All were cases of subacidity with no free hydrochloric acid. These cases were: atrophy of mucosa, 1; chronic gastritis with dilated stomach, 4; ulcer, 6; "nervous dyspepsia," with anacidity, 3; pernicious anemia, 2; gall-stones, 1; cirrhosis of liver with jaundice, 1; cancer of gall-bladder, 1; pulmonary and peritoneal tuberculosis, 3; an interesting group of inflammation of the large intestine (ulcerative colitis, etc.), 5; cancer of the ovary, 1; peripheral neuritis, 1 and fibrinous pericarditis, 1.

One might expect to find lactic acid in the gastric contents in cases with diminished secretion involving the ferments as well as the HCl, with stagnation and perhaps with insufficient absorption (Hammerschlag), yet Riegel found considerable in the gastric juice of cases of cancer without much atony and reported that as the result of his experience of over 20 years the presence of considerable lactic acid practically always means gastric carcinoma. He explained the lactic acid in 1 case without stasis as due to acid-producing organisms retained in the fissures at the base of the cancer.

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[24] Deut. Arch. f. klin. Med., April 24, 1908, Bd. 93, p. 98.

The usual method to test for lactic acid is to examine the gastric contents removed 1 hour after an Ewald breakfast at the same time that one tests for HCl, but this is hardly wise since its formation cannot be as rapid as that of the secreted acid. It is better to wash the stomach out well the evening before and test the contents of the fasting stomach the next morning and then again after a test meal which is free from this acid or bodies which could easily form it.

Lactic acid may be formed by the organisms in the stomach, several of which have been proven to be acid-producers, among them the Boas bacillus; or it may be a normal product of digestion but found when its absorption is diminished (an improbable explanation in many cases); or it may be the product of a specific ferment furnished by the tumor. This is possible since in the autolytic digestion of proteid by ferments from these tumors lactic acid has been shown to arise.

In Osler's cases the fluid removed 1 hour after an Ewald breakfast was examined for lactic acid, hence the per cent. of its incidence will be minimal. Yet it was present in 63% of 137 cases. The figure given by Schiff was 73.5% of a group collected from various writers.

The gross appearance of the gastric contents in cancer cases is of great importance in diagnosis since the meat is poorly and the carbohydrates are well digested. Disturbance of motility is due to mechanical obstruction at the pylorus. On the other hand the motility may be excellent and yet the digestion very poor, which is true of some early cancers not at the pylorus. Strauss claims that this abnormal fermentation is due to bacteria which remain in the clefts of the tumors and so are not removed by lavage.

Tumor fragments are seldom found in the stomach washings. These should be looked for in all bloody masses. Sahli suggests that the stomach be well washed out at night and the fasting stomach again the next morning and this latter wash-water carefully examined. The fragments of mucosa frequently found in the wash water in cases of achylia gastrica may closely resemble fragments of cancer.

Sarcinæ and yeasts are rare in the contents of cancerous stomachs and their presence in large numbers is evidence against cancer. In but 5 of Osler's cases were sarcinæ found. The bacterial flora of the stomach is abundant. The organism which has attracted the most attention is the so-called Oppler-Boas bacillus, which is said to be met with in about 80% of cancer cases (Rutinmeyer), and in almost no other condition. The cultural characteristics of this organism are in dispute, probably since several pass under this title and cultures are seldom made. By Oppler-Boas bacillus we usually have in mind a long, coarse, thread-like bacillus, often in long chains which extend across the field of the microscope. In some cases they are present in such enormous numbers that they even fill the whole field. No spores are seen. The single bacilli are from 3 to 10μ long (6 to 8μ as a rule) and 1μ broad. They have rounded ends and often are slightly bottle-shaped. Some are bent. They do not decolorize by Gram's method and are best seen in stained specimens. It is rather agreed not to report the Oppler-Boas bacilli as present unless such bacilli are present in large numbers. Some of the organisms thus named may be the gas bacillus. The so-called Oppler-Boas bacillus is not anaërobic nor will it grow well on ordinary media, but will luxuriantly if blood or its derivatives be added. This may explain its frequent presence in the cancer stomach in which condition alone blood is almost constantly present. In cancer cases also are the ulcerations and clefts which would hide these organisms during lavage. Here also is there a failure of ferment and of acid secretion and stagnation all of which factors Schmidt considers essential for the presence of this organism. Kaufmann[25] claims that this organism cannot grow if there is 0.02% of free HCl present but can well if the acidity is due to phosphates and lactic acid; but others (Rosenheim) say it flourishes in the stomach in spite of free HCl. It coagulated milk. Kaufmann found this bacillus in 19 of 20 cancer cases, proved that it was a lactic acid builder, and found that it occurred in numbers proportional to the amount of lactic acid present. Most other lactic-acid-forming bacilli are smaller. Other bacilli of similar appearance have been cultivated[26] which adds to the confusion. In a recent case without extreme stasis the gross sediment of the stomach washings was almost entirely composed of masses of these bacilli. If stenosis certainly is present and these bacilli are absent, the evidence is against cancer (unless the stomach has been too well washed out).

The Hopkins series is hardly suitable for purposes of statistics concerning the presence of these organisms, for only the fluids removed 1 hour after an Ewald breakfast (and the stomach had been washed out the evening before) were examined. These organisms were found in only 38% of 55 such cases. In 4 of these lactic acid seems not to have been present.

Heichelheim[27] thinks that in the diagnosis of cancer clots of blood in the gastric contents are very important, especially if they contain many coarse bacilli and the fluid is without free HCl.

Pus is sometimes present in the gastric contents; in fact the largest amount of pus that we have seen in any gastric case was one of carcinoma.

The resorption through the gastric mucosa is much disturbed and the KI test is almost always delayed.

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[25] Centralbl. f. inn. Med., 1904, No. 4.
[26] See Schmidt, Wien. klin. Wochenschr., January 10, 1901.
[27] Zeitschr. f. klin. Med., 1904, vol. liii, p. 447.

Among other tests proposed for the early diagnosis of cancer of the stomach is the tryptophan test of Erdmann and Winternitz[28] which is not constant enough (Sigel in 2 of 15 cases; Glässner in 1 of 2) to be of great value and is positive in other conditions (ulcer, *e.g.*); yet its presence does help.

The presence in the washings of a fasting stomach with good motility of over 0.5 P.M. of albumin (Esbach) is of some, but not great, value. To determine this the stomach is carefully washed out, then a few hours later the tube again introduced and all the fluid possible obtained. The stomach is then washed several times with 400 c.c. of physiological salt solution. The albumin and nitrogen of this gastric fluid are then determined. In all other conditions N = 0 to 16 mgms. but in cancer from 10 to 70 mgms. per 100 c.c.: cancer is probable when N = more than 20 mgms. and there is a definite albumin precipitate by Esbach's fluid. This albumin is supposed to come from the ulcerations of the cancer nodule which furnished this inflammatory exudate.

Gluzinski's test for the relative insufficiency of HCl secretion was proposed especially to indicate a cancer on the bed of an old ulcer. He tested the free hydrochloric acid in the morning on the fasting stomach, 45 minutes after the test breakfast and again 4 hours after a full meal. The test is positive when the stomach fails to respond suitably to the greater stimulus.

Infusoria and *flagellates* especially are sometimes present in very early stages in anacid carcinomatous stomachs. Cohnheim reported 6 cases with Trichomonas and Megastoma entericum present. He thinks this a valuable sign, even the earliest, for the diagnosis of an ulcerating cancer of the cardia or lesser curvature, but not of cancer of the pylorus, since the lactic acid present in these cases would kill them. They are often present in our food. Zabel[29] reported 4 early cases with such organisms present in abundance. Rosenfeld[30] found them in 6 cases, 1 of which he thinks is the first non-carcinomatous case in which they had been found. He thought this was true of still another case, but that proved to be a case of cancer. They are found in the small amount of neutral or alkaline fluid of these fasting stomachs, together with leptothrix threads, long bacilli and spirilla. It is interesting that they cannot be found in other cases of achylia for we certainly must swallow them frequently with our food.

Blood, usually occult, in the gastric contents and stools is a very important, a common (68 of 70 cases) and early sign of cancer, especially in the absence of free hydrochloric acid and when motility is good.

Strauss emphasized the disproportion between the relatively active fermentation and small amount of sediment in case the cancer is not at the pylorus; Reissner, the early increase of chlorides to almost or quite the double and the alkaline reaction of the ashed gastric contents. For Glässner's idea concerning ferments see page 356.

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[28] Münch. med. Wochenschr., 1904, p. 299.
[29] Wien. klin. Wochenschr., 1904.
[30] Deut. med. Wochenschr., 1904.

The " early " diagnosis of cancer of the stomach is unfortunately usually a late one if one means by " early " a time when the patient can be saved by operation. No one feature will help. All evidence must be sought for and carefully evaluated. The chemical signs may be very suggestive in some cases, in others normal, while in some even the reverse of those suggesting cancer. The surgeons insist that the diagnosis should be made long before a tumor is palpable and this should be the aim of the clinical chemist. At present we feel that age and clinical history are more important than chemical examination and this more important than the röntgenological examination. We would never delay operation until the diagnosis was positive but would take a chance if a suggestive subjective history were strengthened by one objective sign, whether chemical, röntgenological or physical.

CHAPTER IV

THE INTESTINAL CONTENTS AND THE FECES

Pancreatic Fluid.—The duodenal contents were formerly obtained by massaging the contents of the duodenum back into the stomach which previously has been washed with a 1% soda solution. The abdomen of the patient lying on his back was massaged from right to left from the costal margin to the parasternal line. The stomach-tube was then quickly introduced and all possible contents removed. Sometimes about 50 c.c. are obtained. To prevent the destruction of the ferments by hydrochloric acid, soda should be added at once. A much more satisfactory method however is made possible by Einhorn's duodenal tube.

The presence of trypsin in the fluid obtained is assumed if fibrin or egg albumin is digested in alkaline medium.

Trypsin—Arthus and Huber's Method.—Fresh fibrin is washed in water, then heated at 40° C. for 24 hours with 2% NaF and filtered. The intestinal fluid plus an equal amount of 2% NaF is mixed with 2 or 3 volumes of the above-described fibrin and kept for some time in the thermostat at 40° C. If trypsin is present the typical crystals of tyrosin will easily be found. Contamination with bacteria need not be feared.

The fat-splitting ferment may be demonstrated as follows: Neutral olive oil is obtained by shaking out olive oil with ether and a little NaOH. The ether extract is shaken out repeatedly with water and the ether then evaporated. It is then emulsified by shaking together 10 parts of oil, 5 of gum, and 35 of water. In each of 4 test-tubes are mixed 10 c.c. of very dilute neutral litmus solution and 5 drops of this emulsion. The fluid to be tested is added, 2, 4, 6 and 8 drops respectively to the 4 tubes, and these are left for a few minutes in a water-bath at 37° C. The presence of lipase will be indicated by the red color in one or more of the tubes.

The presence of diastase is proved if at the end of a few minutes after a little of the fluid has been mixed with a thin starch solution the addition of a drop of iodine solution fails to give a blue color (see page 357).

Brown's method of estimating the functional activity of the pancreas by estimating the diastase of the stools is as follows:[1]

Brown estimated the diastase of the stools in preference to trypsin or lipase since both of these are easily destroyed by bacteria, since trypsin digestion may be simulated by the action of erepsin and by that of the putrefactive bacteria of the intestine and since trypsin must be activated by enterokinase and lipase by bile. On the other hand practically all of the diastase in the bowel is secreted by the pancreas and being a preformed ferment does not require an activator.

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[1] Johns Hopkins Hospital Bull., July, 1914.

Diastase converts starch to maltose the intermediary products being soluble starch, erythrodextrin, achroödextrin and isomaltose. Since constipation lessens the amount of diastase while some purgatives (*e.g.*, senna) increase it, Epsom salts was chosen as the best laxative.

To exclude the salivary diastase liquid food is used to eliminate chewing.

There are many sources of possible error in this method but the following routine will reduce them to a minimum. After a very light evening meal the patient at night is given a high enema. At 7 A.M. the next day he drinks 750 c.c. of milk, and at 7.30 A.M. and again at 8 A.M. he is given half an ounce of Epsom salts. At 8.30 A.M. he drinks a glass of water containing ¼ of a teaspoonful of bicarbonate of soda. All the stools up to 2 P.M. are saved in a vessel containing 2 ounces of toluol and kept on the ice or in a cool room. If less than 400 gms. or cubic centimeters of stool are obtained an enema of a pint of water is given since in the average case between 400 and 1100 c.c. of stool may be expected.

The stool should be examined as soon as possible. It is first diluted up to 3000 c.c. with normal salt solution, stirred until absolutely homogeneous, a portion centrifugalized for 5 minutes and the supernatant, fairly clear fluid used for the tests.

Diminishing amounts of this fluid are put into a series of tubes, 1.8 c.c. in the first, 1.6 c.c. in the second, 1.4 c.c. in the third, 1.2 c.c. in the fourth, 1 c.c. in the fifth, 0.8 c.c. in the sixth, 0.6 c.c. in the seventh, 0.4 c.c. in the eighth, 0.2 c.c. in the ninth, 0.1 c.c. in the tenth, 0.05 c.c. in the eleventh, and 0.025 c.c. in the twelfth. The fluid in each of the tubes is then brought up to 2 c.c. with normal salt solution. To each of the tubes are added 2 c.c. of 1% solution of soluble starch (Kahlbaum). The tubes are now incubated at 38° C. in a water bath for half an hour, then cooled by the addition of tap water to the bath or by placing them under the cool tap, and are then tested quickly with a few drops of *0.1N* iodine solution. The limit is that tube before the one in which the first definite blue color appears. The exact figures would be somewhere between this and the next succeeding tube. Slight variations in the temperature of the water bath and in the reaction of the medium have so little influence on the result that it is not necessary to reduce all the specimens to the same reaction to litmus. If a previous test showed a negative result in the first tube, or if very low readings are suspected, a supplementary series of tubes containing respectively 2 c.c., 3 c.c., 4 c.c., and 5 c.c. of the centrifugalized mixture is used. The unit chosen is the digestion of 1 c.c. of 1% starch solution at 38° C. in half an hour. The lowest normal reading in the series of cases studied was the tube 10, which indicated 60,000 units; if 1 c.c. could digest 2 c.c. of 1% starch solution then 3000 c.c. could digest 60,000. The highest was 240,000.

EXAMINATION OF BILE FROM THE DUODENUM.—Lyon [2] noting the observation of Meltzer that magnesium sulphate in the duodenum produces a relaxation of the sphincter of the common bile duct, proposes the following method of obtaining the bile for the purpose of the diagnosis of hepatic conditions.

The mouth of the fasting patient is rinsed thoroughly with any good antiseptic solution, preferably potassium permanganate, 1 grain to 2 ounces of water, followed by a rinse with a weak solution of zinc chloride. A sterile duodenal tube fitted with any one of the later modifications of the original metal tip, is passed into the stomach. The fasting gastric residuum is aspirated into a sterile vessel, the stomach thoroughly rinsed and the patient, lying on the right side with hips elevated, is given a glass of water to drink while slowly swallowing the tube to the duodenal mark.

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[2] Jour. A. M. A., Sept. 27, 1919, vol. 73, p. 980.

The tube usually passes into the duodenum in from 15 to 45 minutes. (This can be told by the duodenal tug and the character of the fluid aspirated.) When certain that the tube is in the duodenum a barrelful of air is introduced from a 1 ounce capacity syringe to balloon out the duodenal walls from the metal tip (to prevent traumatism of the duodenal mucosa), a connection is made with a sterile aspirating vacuum bottle and gentle aspiration of the duodenum is begun.

The contents of the fasting duodenum should be bile free, pearly gray, of syrupy and stringy consistency, fairly transparent and should have a relatively small amount of flocculent or flaky sediment. In cases of duodenitis the gross appearance, the microscopic sediment and the chemical tests of this fluid differ widely from the normal.

The first bottle is now detached and from 50 to 100 c.c. of a sterile, 25% saturated solution of magnesium sulphate is introduced by means of a sterile syringe or by the gravity method from a sterile container. The tube is attached to the second sterile bottle and aspiration gently started. In from 2 to 10 minutes usually bile is obtained staining light yellow the magnesium sulphate solution still in the duodenum. When the color deepens to a pronounced yellow the material already collected in the second bottle is decanted into a sterile glass container, the bottle is reattached and biliary drainage is continued. The first bile aspirated is, Lyon believes, the bile present in the bile ducts, probably only the common duct. It measures from 10 to 20 c.c. in amount, is lighter yellow in color, is more likely to be transparent and is much less mucoid than the bile seen later. Then the bile suddenly becomes darker, more viscid and more concentrated. In normal gall-bladders it remains transparent, but is more of a molasses-yellow. Lyon believes this bile to be that stored up in and delivered from the gall-bladder. This bile varies in amount from 30 to 100 c.c., in 1 case it was 166 c.c. Lyon believes that the high normal should not exceed 75 c.c. When it appears the bottle is replaced by a third sterile collecting bottle into which the bile is allowed to flow until it is replaced by a lighter yellow, thinner and usually transparent bile which is aspirated much more slowly and intermittently. This, Lyon believes, is bile freshly secreted from the liver. When this second transition appears the third bottle is detached and a fourth sterile bottle attached to collect the liver bile.

In this way Lyon believes we can collect separately bile from different parts of the biliary apparatus and, by receiving them in separate containers, can study each sample separately. This method has already proved itself of great value. The following are a few illustrations of the interesting results recently obtained by this method in the surgical wards of this hospital.

Hosp. No. 10590 is a woman aged 36, admitted Nov. 17, 1920, with a clear history of cholelithiasis. She was admitted with pain in epigastrium, nausea, vomiting, jaundice, clay-colored stools and bile in the urine. The röntgenograms showed a distinct shadow in the gall-bladder region. The bile obtained from the gall-bladder by Lyon's method was definitely purulent. Following this 1 aspiration the pain was at once relieved, the jaundice cleared up rapidly and the patient insisted on her discharge Nov. 22, 1920.

Hosp. No. 10455 was a woman aged 27 admitted Oct. 18, 1920, with a diagnosis of cholelithiasis. On aspirating the bile from the duodenum definite gall-sand was obtained. On operation later similar sand was found in the cystic duct.

Hosp. No. 10371 was a boy 16 years old admitted Oct. 1, 1920, as a case of convalescent typhoid fever with symptoms of cholecystitis. His Widal on admission was positive and his blood culture negative. From the bile obtained by Lyon's method from gall-bladder a pure culture of Bacillus dysenteriæ of Shiga was obtained.

The last case to mention was an out-patient, a woman 26 years old, examined Nov. 6, 1920, for symptoms of cholecystitis. Pus was obtained from the gall-bladder by Lyon's method. In this pus numerous streptococci could be seen in smears but none grew in the culture which gave a pure growth of a diphtheroid organism. This 1 aspiration of bile gave the patient great relief from symptoms.

Motility of the Intestines.—It often is important particularly in metabolism experiments to determine the motility of the intestine in order to separate the stools belonging to a definite period and to determine the presence of a latent constipation. The normal time of passage of food from pylorus to rectum after a mixed meal is from 6 to 20 hours; after milk, from 36 to 48 hours. The motility of the bowels may be determined by watching the progress of a barium sulphate meal by means of the fluoroscope and yet this cannot wholly replace the use of charcoal, lycopodium powder, or 0.5 gm. of carmine. After giving any one of these substances the stools are watched until the black charcoal is seen grossly, or until the characteristic lycopodium spores are seen microscopically, or the red color appears. Allowance should be made for the gastric motility in case the actual time in the intestine is desired. Since the charcoal mixed in a considerable mass of feces may pass unnoticed, lycopodium or carmine are somewhat safer substances to use.

Test Meals.—To study intestinal conditions one should use a test meal with which he has had considerable experience especially with healthy persons. Folin's diet should be used by those who wish to compare their results with his, and he is the only one who has published a complete analysis of the urine of patients on any standard diet.

The patient's diet is as follows (Folin): whole milk, 500 c.c.; cream (18 to 22% fat), 300 c.c.; eggs (white and yolk), 450 gms.; Horlick's malted milk, 200 gms.; sugar, 20 gms.; sodium chloride, 6 gms.; water, enough to bring the whole up to 2000 c.c.; extra drinking water, 900 c.c. This daily ration consists of about 110 gms. of protein, 148 gms. of fat, and 225 gms. of carbohydrate. It contains 18.9 gms. of N., 5.9 gms. of P_2O_5, 3.8 gms. of SO_3, and 6.2 gms. of Cl.

Others prefer for experiments in metabolism a diet of milk alone or of milk and eggs. In following a single patient through several periods of observation any diet will do providing it is constant, but if different patients are to be compared a standard diet is necessary. If the work is to be at all worth while the actual food used must be analyzed. Tables of food composition cannot be used.

McCrudden's[3] method of analyzing food given in metabolism experiments is free from many of the objectionable features of the usual methods. Samples of the foods given the patient are saved for analysis, are all mixed together, in the proportions in which they are given the patient, so that 1 analysis of the mixture as a whole is sufficient. In this way one avoids a separate analysis of each food consumed, the food need not get stale while the experiment is in progress, one can give fresh food and change the diet as often as desired.

In the case of liquid foods the fluids are mixed as thoroughly as possible, a certain volume is given the patient, and the same volume is taken for analysis. A solid food is mixed after being cut into small pieces. The patient receives a certain weight of this and the same weight is reserved for analysis. Only nonhomogeneous foods are excluded.

At the end of the experiment all of this food reserved for analysis is well mixed together, a little HCl added to retain all the nitrogen, as much as possible of its water is removed by evaporation on the steam-bath and the remainder by adding alcohol twice and continuing the evaporation. The food thus dried is next ground up in a grinder and then, since it can more easily be reduced to a powder when free from fat, this is extracted with naphtha. It is now crushed fine, so that it will all pass through a fine sieve and then its volume is reduced by quartering. That is, the food is thoroughly mixed with a large spatula and then made into a little circular pile 2 inches high. This pile is divided into 4 equal quarters. Two opposite quarters are rejected, and the other 2 are well mixed together, made into another little pile, and quartered again. The mixing and quartering are repeated until there is about enough food left for 1 set of chemical analyses.

For the macroscopic and microscopic study of the stools with a view to determining how well the various foodstuffs are utilized, the best diet is that of Schmidt and Strassburger.[4]

Morning: 0.5 liters of milk and 50 gms. zwieback. Forenoon: 0.5 liters oatmeal gruel strained (made from 40 gms. oatmeal, 10 gms. butter, 200 gms. milk, 300 gms. water and 1 egg). Noon: 125 gms. chopped beef (raw weight) broiled rare with 20 gms. butter, 250 gms. potato broth (made of 190 gms. mashed potatoes, 100 gms. milk, 10 gms. butter). Afternoon: As morning. Evening: As forenoon.

This daily diet, which is given for a period of 3 or 4 days, consists of 1.5 liters milk, 100 gms. zwieback, 2 eggs, 50 gms. butter, 125 gms. beef, 190 gms. potatoes, and 80 gms. oatmeal.

It contains about 102 gms. proteid, 111 gms. fat, 191 gms. carbohydrates. Its total caloric value is 2234.

In cases of jejunal fistula it is often important to know how near the opening is to the pylorus. Cushing[5] tied a silk thread to an oyster which the patient then swallowed and which soon appeared at the orifice. By measuring the length of the string this was found to be but 1 foot below the pylorus. This patient drank a glass of milk which began to escape from the fistula in 1 minute and all of which was recovered in 4 minutes.

The examination of the stools deserves much more attention than it receives. Just as the sputum examination is commonly limited to a search for the tubercle bacillus, so that of the feces is now a matter of searching for parasites' ova and much that is valuable passes undiscovered.

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[3] Jour. of Med. Research, 1903, vol. ix, p. 135.

[4] Die Fæces des Menschen, Berlin, 1905. See also Hewes, Boston Med. and Surg. Jour., April, 1909, vol. clx, p. 429.

[5] Johns Hopkins Hosp. Bull., July, 1899.

All that is necessary for this examination are a few tall glass jars in which the stools mixed with water are allowed to sediment, some strainers (colanders) of various sized mesh through which the stool may be ground by a pestle, some large centrifuge tubes and plates half black, half white, similar to those used in sputum examination.

The normal stools consist of the undigested portion of food, bacteria, intestinal secretions, formed and unformed elements from the mucosa, salts and products of digestion. The amount per day varies widely with the diet, but a general average is from 120 to 250 gms.

The bodies of bacteria, the most of them dead, make up about one-third of the entire weight of the dried stools, that is about 8 gms. per day, and contain about ½ the nitrogen of the stools. The stools of some dyspepsias contain more, even from 14 to 20 gms., and strange to say those of cases with chronic constipations less, from 2.6 to 5.5 gms. Strassburger's method of determining the weight of bacteria was as follows: Two cubic centimeters of the stool is well mixed with water and centrifugalized; the organisms will remain suspended while the elements of the food will sediment. The fluid is then decanted, considerable alcohol added to lower its specific gravity, and it is again centrifugalized to sediment the bacteria. The sediment is then dried and weighed. Another 2 c.c. are then evaporated (fresh alcohol being repeatedly added), dried and weighed, to get the total weights of solids in 2 c.c. of stool. Klein [6] used a counting method and takes exception to Strassburger's method and results, but he does not even suggest a guess as to the relative weight of the bacteria in the stool.

The small amount of feces during starvation periods consists of bacteria, the intestinal epithelium, mucus and the intestinal secretions.

Reaction.—The normal stool is usually alkaline in reaction, but may be neutral or faintly acid for the changes on standing are very rapid. The reaction at the surface of a mass of feces may differ from that at the center. If it contains any urine it will of course soon be alkaline. The stools of typhoid or cholera patients are alkaline as a rule, those of patients on milk or starch diet may be very acid.

Frequency.—By *diarrhea* is usually meant the passage of frequent and fluid stools; by *constipation*, infrequent movements of the bowels, associated with symptoms which are relieved by purging. The normal stool is never fluid but frequency is a variable matter and must be judged from the individual standpoint. Some patients are very uncomfortable unless they have 2 movements a day while others normally have but 1 each 2 days. Much more attention has lately been paid to the time required for food to pass through the bowel, for some very constipated persons may have a movement regularly every day (latent constipation) while others badly constipated have several movements a day, hence the old adage, that diarrhea is one of the best symptoms of constipation. In these cases the food collects as scybalous masses in the colon and these by their mechanical irritation and the resulting infections of the colon wall cause the passage of frequent fluid stools often containing fragments of the hard dry masses from above.

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[6] Zeitschr. f. klin. Med., 1903, Bd. 48, p. 163.

DIARRHEA may be due to increased peristalsis, increased intestinal secretion or decreased absorption, and accompanies chronic enteritis, intestinal tuberculosis, amyloid disease, cirrhosis of the liver, cholera, typhoid, dysentery, infectious disease, uremia, etc., as well as various functional nervous disorders.

When the trouble is in the small intestine the movements are fluid and large but not necessarily very frequent; in dysentery they are frequent and scanty.

CONSTIPATION as a chronic condition is the result: (*a*) of careless habits assisted by a sedentary life and a diet lacking in the constituents which stimulate intestinal peristalsis; (*b*) of dilated stomach, constriction of the bowel, chronic appendicitis and chronic cholecystitis, etc.; (*c*) of general muscular atony. Acute constipation occurs in obstruction of the intestine, paralysis of its wall as in peritonitis, meningitis and other conditions causing increased brain-pressure. In acute obstruction due to intussusception, ileus, etc., the frequent stools of bloody mucus which contain no fecal matter may deceive the doctor who does not personally inspect them.

The consistency and form of the normal stool vary considerably, depending on the habit and diet of the individual, and pathologically on the intestinal secretion, absorption, and, especially, motility. The stool may be abnormally too fluid or too solid; when very hard it breaks up into small masses resembling sheep manure, or somewhat larger masses, " scybala," which may be of the size of a walnut and of even stony hardness. Such stools are common after typhoid fever and in some cases on a milk diet. These masses may in the rectum form large accumulations. For the mass of the stool to be of very small caliber does not necessarily indicate a stricture of the rectum since stools of small caliber are common in conditions of anal tenesmus, of inanition and in various nervous diseases. Boas believes that homogeneous, thick, pasty or curd-like stools in which float cylinders of fecal matter about the size of the little finger suggest stenosis of the lower bowel provided the most of the stools are of this character.

The feces are abnormally soft if they contain an excess of water, fat, fruit or of vegetable matter. An abnormal water-content may mean too rapid peristalsis of the colon (which precludes the normal drying of the stool) or an abnormal secretion of water by the colon. For the occurrence of fatty stools and of stools with excessive mucus see pp. 387 and 406. The most common vegetable foods which make the stools soft are cabbage, pears, apples, plums, etc. An easy way to determine whether the softness of a stool is due to fat or to water is to press a cover-glass down hard on a small portion. If when the pressure is relieved the cover-glass springs back and air rushes in from all sides, there is an excess of water; if it stays as pressed, the softness is due to fat. Frothy stools indicate intense fermentation. Such stools often appear acholic (see below).

Color.—The dark color of the normal stool is due to hydrobilirubin. Except in the case of nursing children bilirubin itself is never normally present. The longer a stool remains in the bowel and the longer it is exposed to the air, the darker it is. Certain foods influence its color. Milk makes stools light; meat, dark; cocoa, reddish brown; wines, dark; foods containing chlorophyll, greenish. Several drugs also are important. Calomel sometimes makes stools green (biliverdin); bismuth subnitrite, black (bismuth suboxide); senna, santonin, gamboge and rhubarb, yellow; while the stool containing iron turns dark, even black, after it has stood for some time in the air. A stool which contains digested blood is dark when first evacuated.

A *clay-colored stool* may owe its color to an excess of fat which masks its pigment, or it may be dilute (as in diarrhea), while others owe their lack of color to the action of the organisms of putrefaction which reduce the bile pigments to colorless derivations: but the most important reason for the clay color is the absence of bile in the intestine (as in obstructive jaundice). If the paleness is due to an excess of fat the stool may be extracted with alcohol and ether and the presence of bile demonstrated; if it is due to putrefaction, the color will be restored by exposure to air and the passage of such stools will cease after a dose of calomel. Stools free from bile, the "acholic" stools, are of a grayish white color, have a bad odor and contain much fat.

Bilirubin during its passage through the small bowel is so completely reduced to hydrobilirubin that under normal conditions practically none (except possibly minute traces enclosed in vegetable or soapy masses) reaches the cecum or the ascending colon. The stools contain bilirubin however in cases of diarrhea due to a peristalsis so rapid that the usual reduction cannot occur, and the higher up in the bowel the disturbance begins the more bilirubin will there be in the stools. It will appear in large amounts in the stools, therefore, if this absorption is disturbed and intestinal peristalsis very rapid. This is the case in simple diarrhea. The derivatives of bilirubin may be greatly increased in the stool, even to 400 mgms. in 24 hours, if an abnormal amount of bilirubin enters the bowel with the bile, as in family jaundice.[7]

Since there is considerable bilirubin in the stools in cases of simple colitis some claim that normally much bilirubin does reach the ileocecal valve, but others believe that the so-called colitis practically always is an ileocolitis, or at least that the motility of the ileum is disturbed by a colitis.

Stools containing much bilirubin (and biliverdin) are intensely yellow or green. Sometimes an obstructive jaundice is suddenly relieved and the next movement will be large, soft, of a deep golden yellow color and give an intense Gmelin reaction. In the great majority of cases, however, the presence of bilirubin in the stools is not grossly evident and must be determined by the microscope. For this, Schmidt's test is recommended.

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[7] Tileston and Griffin, Am. Jour. of Med. Sci., June, 1910.

Schmidt's Test.—About 2 or 3 c.c. of the fresh stool, consisting of particles selected to represent as many as possible of its diverse constituents, are covered in a porcelain dish with a saturated aqueous solution of $HgCl_2$ (only the pure salt should be used) and are then ground fine with a pestle so that the mercuric bichloride will mix thoroughly with the stool. The reaction of the mixture should be acid. The dish is then covered and allowed to stand 24 hours at the end of which time the fragments are examined macroscopically and microscopically. The particles stained with bilirubin will have turned green, those with hydrobilirubin, red. The green masses containing chlorophyll must be excluded by microscopic examination.

In diagnosis the green strands of mucus are most important. If large, they are probably from the colon; if small probably from the small intestine if the stool is fluid and especially if the mucus contains the nuclei of many cells the protoplasm of which is digested, or contains cells represented by fat droplets or bilirubin granules. Bile-stained muscle-fibers, connective tissue masses and fat masses are not as convincing since these normally are stained with bilirubin while in the small intestine, and their presence in the stool may mean merely increased peristalsis or catarrh. These would suggest trouble in the small intestine only if the masses of bile-stained mucus also are present.

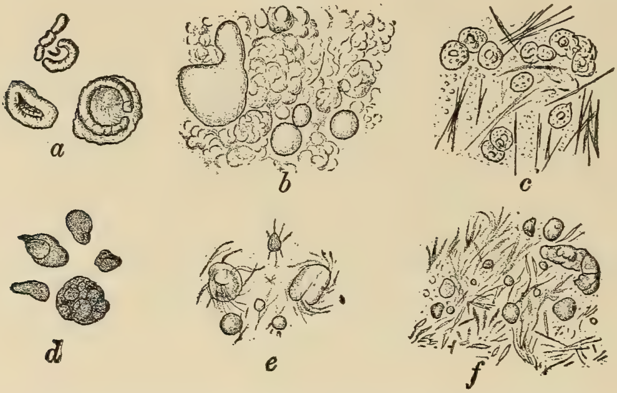

FIG. 72.—Forms of fats and soaps in stools (Schmidt and Strassburger). *a*, soaps; *b*, casein and fat globules; *c*, fatty acid needles and leucocytes; *d*, yellow calcium soap; *e*, fatty acid crystals projecting from fat droplets; *f*, fatty acid and soap needles and scales from an acholic stool.

Bile acids normally are so completely absorbed from the bowel that none appear in the stools.

FATTY STOOLS (Figs. 72 and 73).—If the food contains much fat there always will be some in the stools, either in the form of neutral fat, fatty acids, or soaps. The more difficultly melting neutral fats are present usually as white or yellow lumps, scales or droplets, depending on their melting-point. Fatty acids appear usually as short, delicate, curved needles which occur in such thick masses that the shape of the individual crystal can seldom be made out. The soaps, on the other hand, crystallize out in long needles which are arranged in clusters or fans, or in short plump crystals, or scales. That the most of the needles seen in a stool are soaps may be proven by examining the stool after it has been extracted with ether. The droplets of neutral fat are soluble in ether; the fatty acids are dissolved on warming and in ether, while the soaps are not dissolved on warming nor are they soluble in ether unless they are first split by acid. An easy test for neutral fat is to mix the specimen under the microscope with 1 drop of a concentrated alcoholic solution of Sudan III which has been filtered just before using. The droplets take a color which varies from orange to a blood-red color while the soaps and the fatty acid crystals remain unstained.

Acholic stools usually contain much fat in crystals which are mixed homogeneously with the fecal matter. Such stools have a glistening gray appearance and microscopically contain large numbers of fat droplets and large masses of fatty acid crystals.

In diarrhea the masses of fat needles are sometimes large enough to be seen with the naked eye as minute points. Sometimes, as in pancreas disease, the lumps of fat in a stool are even the size of a nut and of a whitish-gray or a yellowish color like tallow; or the fat may be evacuated as a melted oil which hardens over the cold stool. Indeed, the whole stool may resemble oil. In a recent case of probable cancer of the pancreas the stool looked like a mass of vaseline.

These excessively fatty stools are met with when the diet contains an oversupply of fat. The stools of patients on the olive oil treatment for gall-stones may contain firm lumps of saponified fat which vary in size from that of a pea to that of a hazelnut. Small firm masses of fat are found in the stools after a meal containing fats with a high melting point, as pork, mutton, or tallow.

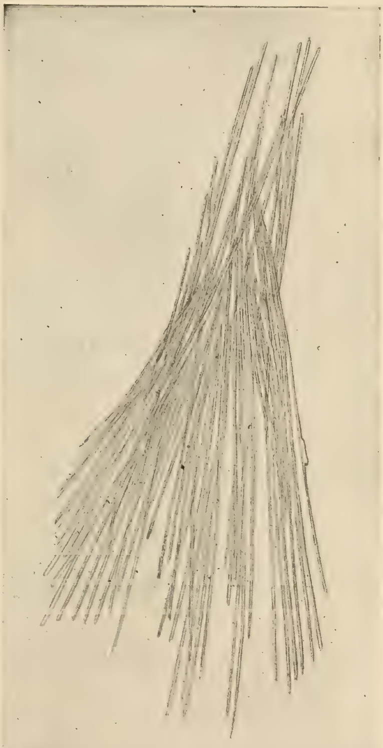

Fig. 73.—A sheaf of huge fatty acid crystals seen often in stools after they have stood a little time. × 400.

Pathologically the stools are fatty when, because of disease of the intestinal mucosa or of the lacteals, the fat cannot be absorbed, as in atrophy of the mucosa, in amyloid disease and in cases with extensive tuberculosis of the retroperitoneal lymph-glands. This last mentioned disease (tabes mesenterica) is the most common cause of very fatty stools in cases without jaundice. In fact, in many doubtful abdominal cases the inspection of the stools alone would suggest this diagnosis. The stool may be fatty in peritonitis and even in simple catarrh preventing absorption. There is a fat diarrhea due to various diseases of the small intestine which is often confused with " diarrhea pancreatica."

In cases of obstructive jaundice from 55 to 78% of the fat ingested will be lost in stools (normally from 6 to 10%). Acholic stools in cases without jaundice may contain large amounts of fat. The cause of this condition is in doubt. Some believe that bile secretion may be temporarily suspended, others that bile pigment is present but has been changed to some colorless forms.

In pancreatic disease fatty stools are common, but to be of value in diagnosis they must be exceedingly fatty. It is, however, also true that in severe pancreatic disease they may not be fatty at all provided the fat ingested had already been emulsified. Müller showed that while normally 84% of the fat is split in the bowel yet if the pancreatic juice be excluded only about 40% will be split. Others consider that 80% may be split but not saponified. The diagnosis of pancreatic disease is exceedingly difficult and cannot be made from the fatty stool alone, yet if all the elements of LeNoble's symptom complex are present (no jaundice, glycosuria, much fat in stools, many fatty acid crystals but no soaps, no hydrogen sulphide, skatol or indol, the stools rancid but not putrid and with few bacteria) one is very safe in assuming the existence of some pancreatic trouble.

ESTIMATION OF FATS AND SOAPS.—The stool is first evaporated over the water-bath until of a semisolid consistency, then mixed with about 50 c.c. of absolute alcohol, again evaporated and this repeated until it is perfectly dry. A certain amount is then powdered, dried at 100° C. and weighed. This is rubbed up with sand and extracted for from 8 to 10 hours with ether. The ether residue is washed with warm water, dried in a desiccator and weighed. It consists of neutral fats and fatty acids.

To isolate for weighing the neutral fat the residue is again dissolved in ether and shaken out with a dilute soda solution, which will remove the fatty acids.

The fatty acids are determined by dissolving a weighed amount of the ether residue in alcohol and ether and then titrating this with an alcoholic solution of potassium hydroxide, phenolphthalein used as indicator.

For the determination of the soaps, some of the ether residue is boiled with acid alcohol, dried, extracted again with ether and the free fatty acid in this extract titrated as above.

If one wishes to determine at once the neutral fats, the split fats and the soaps, the stool is first boiled with acid alcohol before it is extracted with ether (Müller).

Mucus.—The stools always contain a certain amount of mucus (Boas). This is, however, seldom seen even microscopically and must be tested for chemically. Any visible mucus is somewhat abnormal. The mucus present is pure mucin, hence can be clouded by acetic acid. A specimen of stool is rubbed up with water, an equal amount of lime water added, it is allowed to stand for several hours and then acetic acid is added. A cloud will indicate mucus.

Mucus is increased physiologically by hypersecretion, and forms a glassy or cloudy coating over hard fecal masses evidently to protect the mucous membrane against these. It may also be present after an active purge. This mucus is poor in cells.

Mucus from the small intestine is intimately mixed with the stool and hard to isolate. In diarrhea these small flecks can be picked out with a needle and if the stool be solid they appear as shreds or lumps which never are bile-stained. Such small flakes resemble those from the stomach. They are cloudy since rich in the detritus of digestion and in cells the bodies of which often are well digested or represented by masses of bilirubin granules and crystals. The so-called " sago granules," or " spawn-like " masses of mucus described by Virchow, are, Boas thinks, very rare. Mucus may be seen microscopically in the stool as small transparent lines and masses. The minute yellowish or greenish mucous granules or " islands " emphasized by Nothnagel as indicating catarrh of the small intestine are, Boas and Schmidt consider, exceedingly rare and consist more of albumin than of mucus. Much mucus is present in the stools in cancer of the rectum with stenosis (see also page 423).

Some stools consist chiefly or entirely of mucus, which is glistening and jelly-like and is evacuated sometimes in masses resembling " frogs' eggs," or in strips sometimes over a foot long suggesting to the uninitiated tapeworms or pieces of bowel. This mucus comes from the large bowel, especially the sigmoid flexure. Its evacuation is usually preceded by a colic which often is very severe and which leads to serious error in diagnosis. Some writers have attempted, but in vain, to distinguish between *enteritis membranacea* (a mildly inflammatory condition) and *mucous colitis* (a secretory neurosis). Excluding the cases with a pelvic tumor pressing against the rectum this condition is in large measure a secretory neurosis although the most of these patients have been constipated for years. Over 80% of these patients are women. Some pass mucous stools daily for a week at a time, some pass 1 a week or 1 a month, others pass them still more seldom. The relation between these stools and intestinal sand is interesting (see page 400). In general the student should be warned against advising operation on any patient who passes mucus in the stools if pain is the chief indication for the operation.

Blood.—It is of course necessary to exclude the blood from raw meat and that due to hemorrhage from the mouth, nose, lungs and vagina. The presence of blood may be suspected from the red or tarry black color of the stool (although even 5% of blood in the stool may pass unnoticed) or it may be found microscopically or proven by chemical tests. Its distribution in the stools is important; fresh blood covering a formed stool indicates hemorrhoids; if evenly distributed with the food matter, it indicates hemorrhage into the stomach or upper bowel, providing the stool is solid. Tarry blood is usually from the stomach and duodenum; blood from small intestine (as in typhoid fever) is usually red. Bloody mucus suggests dysentery. Stools of blood and mucus without fecal matter suggest intussusception and volvulus. Traces of blood are continuously present in the stools of patients with malignant diseases located anywhere along the alimentary tract; traces present during definitely limited, usually short, periods may come from peptic ulcers, seldom from tuberculous ulcers. In typhoid fever blood may be detected chemically in the movement which precedes the bloody stool. In general chronic passive congestion there usually is blood in the stools, but not often in cases of cirrhosis of the liver with portal obstruction. It is present also in some cases of gastric hyperacidity and usually in poisoning by mercury.

THE TESTS FOR OCCULT BLEEDING have their greatest value in the diagnosis of malignant diseases of the alimentary tract and in the differential diagnosis between peptic or duodenal ulcer and nervous gastralgia.

Guaiac Test.—About 3 gms. of feces are thoroughly mixed with a little water until fluid, then about ⅓ volume of glacial acetic acid added and it is then extracted with 10 c.c. of ether. To avoid emulsifying the mixture the tube should be slowly inverted, not shaken.

A piece of clear brown gum guaiac (any green portions should first be removed) about the size of a cherry is crushed, and dissolved in a test-tube half full of alcohol. This tincture should have a light cherry color.

To about 5 c.c. of the extract of the stool are added about 0.5 c.c. of the guaiac tincture and then 1 c.c. of commercial (3%) hydrogen peroxide, or an equal amount of old oil of turpentine. Fatty stools should first be extracted with ether to remove the fat. In the presence of blood a blue color quickly develops which first deepens and then fades to a pale green.

If the diet contains raw meat this test may be positive, but seldom, if ever, if the meat is cooked. Eggs never disturb the test. The fact that the acid stool has been extracted with ether eliminates the most of the disturbing factors, such as milk, pus, saliva, spices and all drugs containing iron.

The Aloin Test of Klinge and Shaer is very delicate. As a preliminary step all foods containing hemoglobin and chlorophyll and all drugs should be discontinued and the patient put on a milk, bread, eggs and fruit diet. Much fat with the food should be avoided. The diet period is to be limited by charcoal, not by carmine.

The stool if very dark in color is rubbed up with 10 volumes of alcohol, this filtered off to remove the urobilin and the stool dried on the filter paper. About 5 gms. are then digested for 1 or 2 minutes with 5 c.c. of ether. From 1 to 1.5 c.c. of oxygenated turpentine are then superimposed and 0.5 c.c. of fresh 3% aloin solution (0.3 gm. powdered aloin is dissolved in 10 c.c. of 60 to 70% alcohol). A fine red ring appearing in from 3 to 5 minutes at the line of separation indicates blood if the patient has been on the above-mentioned diet for several days and if the test is confirmed by several examinations.

Benzidin Test.—Method of Schlesinger and Holst. A piece of feces about the size of a pea is thoroughly mixed in a test-tube about ⅓ full of water, using a clean glass rod, and the mixture brought to the boiling point over the free flame to destroy any enzyme present. While this is cooling a fresh approximately saturated solution of benzidin is made by dissolving in a clean test-tube a knife-point full of benzidin purissimum (Merck) in about 2 c.c. of glacial acetic acid. One now mixes in a clean test-tube 10 or 12 drops of this fresh benzidin solution and from 2½ to 3 c.c. of commercial hydrogen peroxide (3% H_2O_2), shaking the tube lightly. If a green or blue tint appears in this reagent it cannot be used. If the reagent is satisfactory, a few drops of the boiled suspension of feces are added. If blood is present a beautiful green, bluish green, or blue color will appear within 2 minutes which changes later to violet. The depth of the blue and the rapidity with which it appears will depend on the amount of blood present. Only a definitely green or blue color is positive; faint tints are to be disregarded.

This test performed in this manner requires less than 5 minutes and is by far the most sensitive of all. When negative it excludes the presence of even minute traces of blood. If positive, it should be confirmed by the guaiac test, which is safer if meat has not been entirely excluded from the diet. In all tests for blood 2 or 3 portions of the same solid stool should be used, as one may contain considerable blood and another none.

Vaughan[8] recommends for clinical use Wagner's dry benzidin test for blood which he has slightly modified. A knife-point of powdered benzidin (an amount the size of a match head) is mixed in a short tube with 2 c.c. of glacial acetic acid and 20 drops of a 3% solution of hydrogen peroxide. This reagent will remain good but for 2 or 3 hours. A particle of feces the size of a match head is picked up on a toothpick, spread somewhat on a glass slide and covered with 1 or 2 drops of the reagent. The presence of blood will be indicated by the appearance in 5 seconds of a greenish blue color which persists for a minute or more and then fades. It is best seen if the slide is viewed against a white background.

Instead of a slide a piece of glazed paper (a calling card) may be used and has the advantage that it need not be cleaned up afterwards.

This test is not, says Vaughan, too delicate for clinical use and is not interfered with by meat fibers, pus and the usual drugs and foods, but would be positive if the diet contains considerable raw meat.

Pus.—Very rarely is there enough unaltered pus in the stools to be recognized macroscopically and when there is it always indicates the rupture of an abscess (*e.g.*, appendix abscess) into the intestine. On the other hand the contents of even large abscesses may be passed unrecognized, so altered may the pus be by digestion and decomposition. Pus-cells are not recognizable microscopically if mixed with food, but are when enclosed in masses of mucus. The few scattered pus-cells seen in most mucus have no significance (although the mucus may have), since the normal intestinal mucosa contains many leucocytes which wander into the lumen of the bowel. Mucus containing an unusual number of single pus-cells may mean catarrh; that containing masses of these cells means ulcer or (especially if it contains blood also) cancer.

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[8] Jour. of Lab. and Clin. Med., March, 1917, vol. ii, No. 6.

Muscle and Albumin.—Muscle fibers can be found in the stools of all normal persons on a meat diet. The degree of their digestion may be estimated by their appearance. Some show beautiful cross, others only a longitudinal striation, while still others can be recognized as muscle fibers only by their shape, size, and yellow color due to their affinity for bilirubin. Under normal conditions these fibers occur singly. To find bundles of muscle fibers means a pathological increase. In diabetes and diarrhea they appear in the stool in large numbers, even in masses visible to the naked eye. The question whether or not there is a pathological increase of the muscle fibers in a solid stool is best answered by noting their size, shape and striation. In general it may be said that under normal conditions one finds none in bundles and none with the cross striation well preserved.

The condition of *lientery* (the presence in the stools of grossly visible particles of undigested food) is, of course, present in cases with a gastro-intestinal anastomosis, but also in a great variety of other conditions with loose bowel movements.

The presence of an abnormal amount of muscle-fiber in a fairly thick or solid stool and without diarrhea is known as *azotorrhea*. This is suggestive of, but not at all conclusive of, pancreatic disease.

Milk curds and **masses of coagulated albumin** may be found in the stools of infants, but also of adults on a pure milk diet or one containing much coagulated egg. Of the so-called milk curds found in infants' stools the tougher ones consist of casein, while many of the softer ones are almost pure fat.[9]

Starch.—It is seldom that well-preserved single starch granules are seen in the stool of an adult, yet vegetable masses enclosing masses of such granules are common enough. The presence of the former indicates either diarrhea or hyperacidity. Starch granules are never bile-stained. In achylia pancreatica the starch may not be increased in the stools since the bacteria will break it up. The lack of bile also may cause no increase. The iodine test will indicate the extent to which starch granules have been digested, a blue color indicating the unchanged granules; red, a slight digestion.

Carbohydrates.—To detect carbohydrates in the stool Strauss recommends the following method: From 2 to 3 gms. of the dried stool, which should not contain mucus or lactose, are heated for an hour and a half in a flask with a return cooler, with 100 c.c. of 2% HCl. The contents of the flask are then cooled, neutralized quite accurately with sodium hydroxide, filtered through an asbestos filter, washed with water, if necessary filtered a second time and the filtrate brought up to 200 c.c. Fifty cubic centimeters of this are poured into a 300 c.c. beaker and the sugar determined quantitatively. This amount multiplied by 0.94 equals the amount of starch originally present. To test the stool qualitatively for glucose it may be boiled with water and the filtrate then tested with Fehling's or similar solutions. The albumin should first be precipitated with the acetate of lead, the lead then removed with CO_2 and the filtrate tested. It is seldom that any glucose is found in a stool which has not been boiled with acid.

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[9] Jour. A. M. A., 1910, p. 372, and Talbot, Arch. of Pediatrics, Dec., 1909.

Ferments.—The ferments in a stool may be extracted with glycerin and the digestive power of the extract tested. Leo's method is to mix the feces with chloroform water until they form a thin pasty mass and to suspend in this a gauze bag containing from 2 to 5 gms. of finely divided, previously boiled blood fibrin which will absorb the ferments. In 24 hours the bag is removed, the fibrin washed a number of times with water and then tested for the ferments. To test for trypsin, a little of the fibrin is placed in a 1% solution of soda and left in an incubator for a few hours and then filtered. A positive biuret will indicate the presence of trypsin. For diastase a little of the fibrin is placed in a thick starch solution in the thermostat and in a few hours its filtrate tested with dilute Lugol's. If the first drops do not give the blue color of starch then some has been digested. Normally the ferments are destroyed or absorbed in the intestine, yet each may be present in the stools in cases of diarrhea.

Microscopy of Stools.—For the microscopical examination of the stools care must be taken in the selection of the particles to be examined, for a random search usually yields little. To examine for parasite eggs, etc., it is best to mix the stool with water and centrifugalize it or allow it to sediment in a tall jar since these will sediment rapidly. Mucous particles from the fresh, still warm stool should be chosen if protozoans are the object of search. In searching for blood especially it often makes a considerable difference whether the right particle is chosen or not.

EPITHELIAL CELLS.—Squamous epithelial cells are often found in the mucus on the surface of the stool, and come from the anal regions. Great numbers may be present in cases of rectal cancer and of proctitis.

Cylindrical epithelium is the commonest form of epithelial cell found. In some cases of diarrhea they are so numerous that these cases are grouped under the title "desquamative catarrh." The cells are easiest found in the mucus obtained by lavage of the rectum and sigmoid. They show all grades of degeneration. Some, even the goblet-cells, are fairly well-preserved, others are very fatty, while still others are merely remnants of cells.

CRYSTALS.—*Triple phosphate crystals*, irregularly formed as a rule, and typical calcium phosphate crystals are usually present. In addition are found calcium salts of still unknown acids which take the form of irregular, oval, or circular masses, sometimes fissured, sometimes concentrically striated. These are always bile-stained. The masses of calcium soaps are frequently numerous (see Fig. 65).

Cholesterol is often present in the stool, but rarely in typical form and must be demonstrated by chemical methods. *Charcot-Leyden crystals* (Fig. 74) are met with in a great variety of diseases. If present in abundance they always suggest the presence of some animal parasite, it may be any from the harmless oxyuris to the pernicious uncinaria. See Fig. 87.

Bismuthous suboxide occurs as black, irregular rhombic crystals after the administration of bismuth subnitrate (see Fig. 76). Hematoidin crystals are met with but are very rare.

Remnants of undigested food make up the most of the microscopic picture. The student's attention is especially attracted by the thorn-like spines (see Fig. 77) from various fruits and berries; the spiral cells from the veins of leaves; cells with thick cellulose shells, some resembling soap masses, others very like parasite eggs; the elastic tissue from meats, etc. The list is too long and varied to allow enumeration (see Figs. 75, 76).

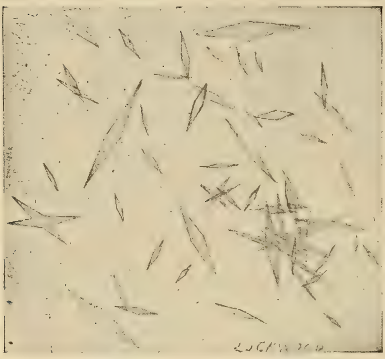

FIG. 74.—Charcot-Leyden crystals from the stools. × 400.

Macroscopical Examination—GALL-STONES.—To find gall-stones in the stools (and a careful search should be continued for 15 days after an attack of colic) the stools are well mixed with water and then rubbed through a sieve. The failure to find a stone after a quite typical attack of colic may, granting the pain was due to a gall-stone, have several explanations: the stone may not have entered the cystic duct, but fallen back into the gall-bladder; it may have remained in the ampulla of Vater; or, it may have disintegrated in the bowel, as some, perhaps all soft stones without a hard rind, do.

Gall-stones vary in size from tiny concretions to others as large as hen's eggs. The single stones are usually spherical and have a rough surface, but when multiple have smooth deep facets. When fractured they may be seen to consist of concentric layers. (Every suspected mass in the stool should be fractured since enteroliths and fragments of bone, as, *e.g.*, a bird's vertebræ, sometimes closely resemble gall-stones.) Gall-stones are composed chiefly of cholesterol and the calcium salt of bilirubin (sometimes also of biliverdin, bilihumin, bilicyanin), together with traces of calcium carbonate. Some rare stones consist of almost pure cholesterol. These are glistening gray in color, rough and soft.

For analysis the stone is dried and powdered. This is necessary even in the case of small stones since they have a mucous coating which prevents the action of the solvents. This powder is then dissolved either in alcohol and ether, in which case the cholestrol crystallizes out as the ether evaporates, or in boiling alcohol from which the cholesterol will precipitate on

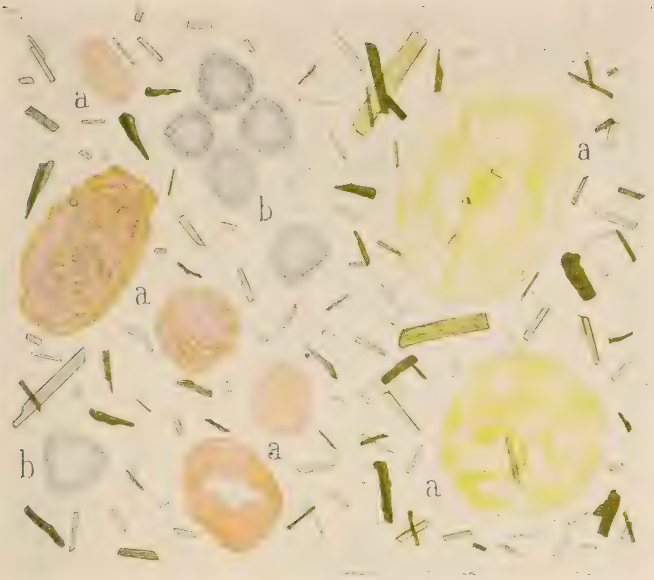

Fig. 75.—*a*, Vegetable cells in stools, resembling parasite eggs. The cell on the left is an unfertilized ascaris egg; *b*, lycopodium spores; the crystals are an iron salt. × 400.

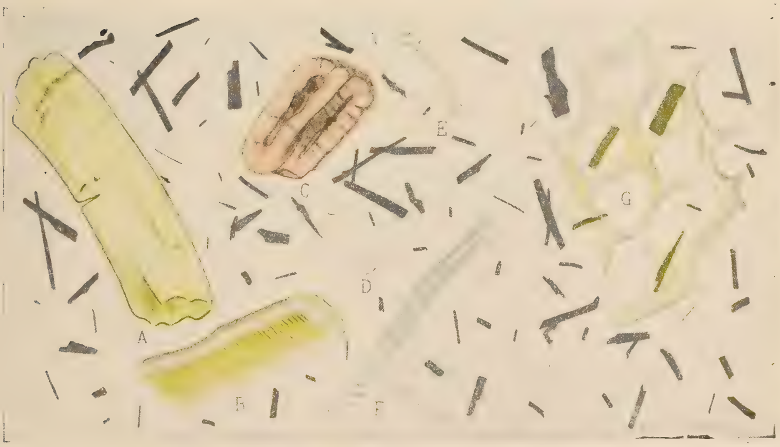

Fig. 76.—Cells in stools. *A*, *B*, muscle fibers; *C*, *D*, vegetable cells; *E*, *F*, spinal fibers from a piece of lettuce; *G*, cellulose framework of vegetable tissue. The crystals are of bismuthous oxide. × 400.

cooling. After the cholesterol is extracted the residue is treated in the cold with very dilute KOH solution which will extract the bilirubin in a yellow solution which will give Gmelin's test. This solution will be blue if bilihumin is present.

Pseudo Gall-stones.—A little care would prevent the many mistakes which result from a failure to distinguish between true and pseudo gall-stones. Each suspected concretion should at least be fractured and many tested chemically. The more dangerous pseudo gall-stones are masses of vegetable tissue, seeds of fruits, pieces of bone, enteroliths, and masses of fats and soaps of high melting point. Olive oil won its great reputation as a medicine to remove gall-stones since if administered in large doses the stools may contain even hundreds of firm masses of soaps.

Gall-sand.—The sand-like sediment often called gall-sand and so abundant in some stools, probably does not come from the gall-bladder. Genuine gall-sand would probably disappear in the bowel, and even though it did not the bile could not explain the large quantities found in some stools (Naunyn). And yet gall-sand may be recovered from the duodenum by Lyon's method (see page 385) and it certainly explains some cases of paroxysmal hyperchlorhydria.

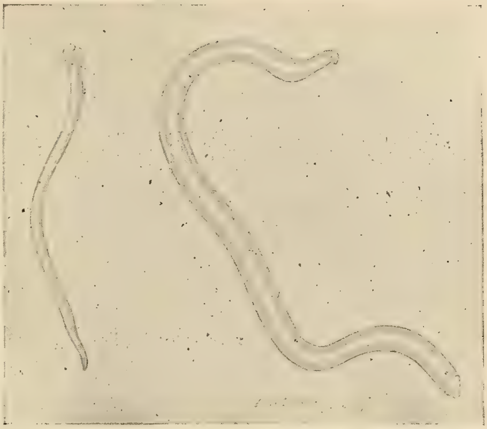

Fig. 77.—Spines forming the "down," that on the right of a raspberry, on the left of a quince. These are often taken for the embryos of parasites.

A private patient, a physician 45 years old, had, for over 20 years, suffered quite frequently from "heart-burn," often paroxysmal and particularly apt to waken him at 2 A.M., at which time he often would induce vomiting thus obtaining complete relief. He could always gain relief also by taking soda. The diagnosis made was gastric ulcer, although the röntgenological examination showed the emptying time of the stomach normal or even shortened.

On one occasion he had pain, not very severe, suggesting a gall-stone in the cystic duct and suggested at once operation. The gall-bladder and its contents appeared at first normal but more careful examination revealed fine granules of gall-sand. No evidence of ulcer was found. The gall-bladder was drained. This operation was followed by great relief.

Pancreatic stones are seldom if ever found in the stools. Those in the pancreatic duct are white, usually single and consist chiefly of calcium carbonate.

Enteroliths.—By enterolith is meant a hard lump of food or mass of hardened feces encrusted with inorganic salts. Enteroliths are seldom passed in the stools. Their chief importance is in connection with appendicitis, although the little hard lump of merely dried feces often found in the appendix seldom is an enterolith.

INTESTINAL SAND.—Intestinal sand is the name applied to the sediments of small, stony hard concretions about the size of genuine sand grains which occasionally is found in the stools. Concretions over 2.5 mm. in diameter should not be called " sand." It sometimes appears in considerable quantities, even half an ounce in 1 stool. Its passage may be an incident of a nervous crisis and may be preceded by considerable pain. Most of the cases reported have been neurasthenic patients with a history of mucous colitis. (This is not surprising, since we seldom examine the stools of normal persons.)

Many specimens of so-called intestinal sand have proved to be pseudo-sand made up of the seeds of berries, bananas, granules from the seed case of pears (these vegetable masses can be easily recognized by studying the cross-section of a granule), concretions of altered blood-pigment, bile-pigment and concretions of medicine, as salol. In other cases the " sand " is genuine, *i.e.*, is quartz swallowed with the food. The best article on this subject is that of Myer and Cook,[10] who believe that most of the sand is vegetable matter. They cite a case in which the granules which were of stony hardness proved to consist of resin and tannin, products of the digestion of the milk-cells of bananas. But apart from these cases one does in rare instances meet with a condition which would seem to be a secretory neurosis which deserves the name " gravel-forming enteritis " (Eichorst).

Chemical analysis of true intestinal sand has shown that it consists of the phosphates and carbonates especially of calcium, but also of magnesium, iron, etc.; while in some of the granules calcium sulphate predominates.[11] Practically all, however, contain some organic matter, as bacteria, fat, cholesterol and urobilin. The granules are described as spherical or angular in shape, very hard, from 0.15 to 2.5 mm. in diameter, and often of a reddish-brown or green color.

We have seen several cases of pseudo-sand and 2 very good cases of, we believe, real intestinal sand. In 1, a young boy ill with an indefinite nervous disorder, such large amounts of fine granules were occasionally passed that they made up a very conspicuous constituent of the stool. The other patient was a young woman with an intestinal neurosis. In this case the granules seemed to be plugs of cells impregnated with carbonates. The nature of these cells could not with certainty be determined, but they were the size of columnar epithelial cells. We have found a few such granules in simple diarrheal stools and the further study of such cases may determine the nature of these interesting bodies.

Bedford [12] thinks that in his case there was a definite relationship between intestinal sand and gout and tophus formation.

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[10] Am. Jour. Med. Sci., March, 1909.

[11] See also Garrod, Lancet, March 8, 1902, and Eichorst, Deut. Arch. f. kl. Med., 1900, Bd. 68, page 1.

[12] Lancet, July 26, 1902.

Tumor Fragments.—Tumor fragments and adenomatous polyps (which may develop as an independent disease or grow in the neighborhood of cancers or ulcers) may be met with in the stools as firm fragments of a grayish-red color and tough consistency. These have their origin in the rectum or colon, or even higher. They are very easily overlooked unless the stool is thin. They may be recognized from the general arrangement of the nuclei in the microscopic sections; the fine details all will have been lost.

Intestinal Parasites.—Protozoa.—Rhizopoda.

Entameba Dysenteriæ or Histolytica.—The protozoon formerly called Ameba coli (and still so called by many), is now generally admitted to be the cause of "amebic dysentery," a colitis characterized by its very chronic course, a tendency to relapse and the frequency with which it is associated with abscess of the liver. When Shiga's bacillus was first discovered it was thought by many to be the cause of the so-called amebic dysentery and Ameba coli was considered a secondary invader; but amebic dysentery and bacillary dysentery now are recognized as two distinct diseases.

The amebæ are often abundant in the masses of bloody mucus passed in the stools of patients with amebic dysentery, although many more are in the ulcers which undermine the mucosa of the colon and of the ileum and in the sinuous fistulæ which radiate from these ulcers for long distances under the mucous membrane. They are found also in the contents of but more easily in the walls of the liver abscesses which so often complicate this form of dysentery, and in the sputum of patients through whose lungs such abscesses have ruptured.

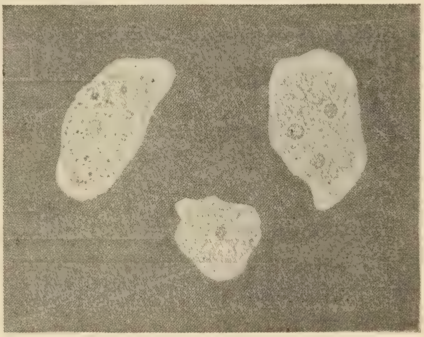

Fig. 78.—Ameba coli (Entameba dysenteriæ), common form. × 400.

Entameba dysenteriæ (Fig. 78) is a rhizopod which varies in diameter from 8 to 50μ. It has a clear hyaline ectosarc, seen best in the pseudopods, and a finely granular endosarc which usually contains some of the parasite's ingesta (red blood-cells, leucocytes, bacteria, epithelial cells and particles of food) and often 1 or more vacuoles which do not pulsate. Its spherical nucleus, about 6μ in diameter, is sometimes, although rarely, seen in the living parasite. To demonstrate the nucleus one kills the organism with corrosive sublimate and stains it by appropriate methods.

The organisms found in various cases of this form of dysentery do not all look just alike. Some differ so much in appearance from those in others that the temptation is ever present to describe different varieties of the parasites.

In the fresh stool, if the stage of the microscope is not too cool, this parasite is ameboid. Some move slowly, others so fast that they are with difficulty kept in the field of vision, while others merely project 1 of their pseudopods. They are very sensitive to an acid reaction of their environment.

These amebæ multiply by simple division. Resting, resistant forms, or "encysted amebæ," the nuclei of which have divided into several nuclei each in the center of a clump of protoplasm, have been described. It is probable that the infection of a new host is effected by these encysted forms.

Since it is almost impossible when examining a stool to distinguish resting amebæ from swollen degenerated epithelial cells it is of great importance that the diagnosis be based solely on cells which unmistakably project pseudopods and never on those the motility of which is doubtful no matter how closely they may resemble amebæ (although quiet cells resembling amebæ which contain red blood-cells, leucocytes and bacteria probably are amebæ).

The stools should be examined while fresh and while warm for the parasite is sensitive to cold. If the specimen is kept warm the parasite will remain active for even 24 hours. (The common mistake of overheating the stool should be avoided.)

In choosing particles for examination one selects, if present, flecks of mucus and in the absence of these the liquid part of the stool. If the stools are firm the patient is given a dose of salts and the next liquid stool examined; or, a solid fragment of stool may be mixed with normal salt solution and then examined. One of the best methods of obtaining this parasite is to pass a rectal tube and examine the little fleck of mucus which the edge of the eye of the tube will scrape from the mucosa. The parasites if present at all will usually be found in the mucus in clusters of scores to a field.

During an acute exacerbation of a case of chronic amebic dysentery the patient usually passes 5 or 6 stools a day, seldom more. The stools are then loose, not watery, and contain mucus which is usually blood-stained and generally mixed with some free blood. During the periods of constipation which separate these periods of diarrhea the amebæ can often be found in the firm stools. Some cases of amebic colitis give no history of dysentery but rather of years of constipation. This is particularly true of the cases complicated by amebic liver abscess. Other cases have throughout their entire course acute bowel symptoms, the frequent passage of small masses of blood-stained mucus. Still other cases are latent and cause the patient few, if any, symptoms, although the amebæ in the stools may be numerous.

Years before Lösch described Ameba coli as a pathogenic organism it was known that amebæ were often present in the stools of persons who had no dysentery or ulcerative disease of the bowel; in simple diarrhea, in typhoid fever, in acute and chronic enteritis, colitis and proctitis, and even in the stools of healthy men. In 1893 Quincke and Roos described: Ameba coli (Lösch), 15 to 25μ in diameter (encysted forms 10 to 15μ), pathogenic to men and to cats; Ameba coli mitis, which is 25 to 35μ in diameter, which may ingest bacteria but never red blood-cells, and which is slightly pathogenic to man, causing a mild enteritis, but not at all pathogenic to cats, and Ameba intestini vulgaris, similar in appearance to Ameba coli but not pathogenic.

The best contribution to this subject is that of Schaudinn [13] who separates Entameba coli from Entameba histolytica, the former the common harmless variety, the latter the pathogenic form causing dysentery.

Craig claimed that Entameba coli can be found in the stools of 65% of normal persons after a dose of Epsom salts; that it is somewhat smaller than Entameba histolytica (10 to 20μ in diameter), is less actively motile, has less difference between endosarc and ectosarc (the latter is less refractile, the former has less demonstrable structure), vacuoles are less common and that the nucleus is more distinct than in the pathogenic variety. What is more important the pathogenic variety shows no encysted stage, but multiplies by sporulation. (For more details, see Craig.[14])

The problem of non-pathogenic amebæ may be of interest to the zoölogist, but the medical man should consider as possibly pathogenic every ameba he finds in the stools. Musgrave believes that any ameba which has been harmless may become pathogenic. Of the 300 persons in Manila whom he examined, 101 were infected with amebæ. Of these, 61 had dysentery and the other 40 had no sign of the disease. During the next 5 months, however, every 1 of these 40 developed a definite dysentery.

Amebæ may be cultivated, but it is with difficulty and only with certain bacteria. These cultures withstand drying for 15 months.

FLAGELLATA.—Of the flagellata only certain of the enflagellata are important in human pathology, especially the protomonadina and the polymastigina. Flagellated rhizopods and lower plants may be found but have no importance.

Polymastigina.—These are flagellata with 3 equal or from 4 to 8 unequal flagella inserted at different points. Some have also an undulating membrane, often mistaken for a row of cilia. Of these 2 groups the Trichomonas and Lamblia are of importance.

Trichomonas.—This is a family of pear-shaped organisms, rounded in front, pointed behind, with at the anterior end 3 or 4 equally long flagella which often are united at their base. The undulating membrane, which is usually present but not always seen, begins at the anterior pole and extends obliquely backward. The nucleus is anterior and behind it are 1 or more vacuoles which do not pulsate. A flagellate about to extrude its flagella may resemble an ameba since the protrusions of the cell membrane resemble pseudopods.

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[13] Arbeit. a. d. Gesundheitsamte, 1903, xix, p. 563.

[14] Am. Med., May 27 and June 3, 1905.

Trichomonas Vaginalis (Donné).—This parasite (Fig. 79) is from 15 to 25μ long and from 7 to 12μ broad. Its posterior end is drawn to a thread. Its cuticle is thin and its protoplasm free from granules. It has usually 3 flagella of equal length, which sometimes seem united at the base, and an undulating membrane the edge of which has probably been mistaken as the fourth which some describe. This parasite is found in abundance in the acid secretion of cases of vaginitis.

In the intestine various similar flagellates have been described under such names as Protoryxomyces coprinarius, Monocercomonas hominis (Grassi), Cinænomonas hominis (Grassi), Trichomonas hominis (Grassi), Cercomonas coli hominis (May) but all of these are now considered to be identical with Trichomonas vaginalis, which parasite can live in the urethra, the large and the small intestine, the stomach, the mouth, and even lung cavities and in the Dietrich's plugs. It owes its name to the fact that it was discovered first in the vagina. It has long been a debated question whether these parasites were harmless or not; whether they caused diarrhea or merely aggravated a trouble already present. It is now considered pathogenic and the adequate cause of even a severe diarrhea.

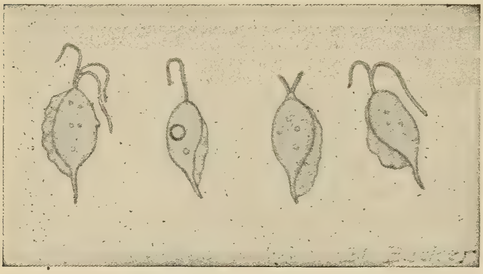

Fig. 79.—Trichomonas vaginalis.

J. W., aged 58, male, Med. No. 10101, was admitted Aug. 11, 1920, with a severe and persistent diarrhea of 3 years' duration. One year ago a physician discovered that his blood Wassermann was 4 plus and since that time he has had thorough anti-syphilitic treatment with both neo-arsphenamine and mercury, but the diarrhea has been uninfluenced by the treatment and the stools now are as frequent as 10 to 20 a day. Examination of the stools revealed the Trichomonas intestinalis in large numbers, 4650 per c.mm. in 1 specimen. (In this count Dr. Hahn considered only definitely motile organisms. Had he included those not moving the count would have been at least 10% higher.) There was no blood and little mucus in these stools. Examination of the mucosa of the colon for amebæ was negative. Gastric analysis showed a complete absence of free hydrochloric acid, pepsin was present, but no blood or lactic acid. Yeast cells were numerous. The olive tip of the Einhorn tube did not pass through the pylorus in 3 hours. The stomach practically emptied itself in 1 hour of a test meal consisting of 1 slice of toast and 12 ounces of water. Fluoroscopy showed very active gastric peristalsis and signs of pylorospasm; great quantities of gas in the colon; a large, long, fixed appendix, slightly segmented. The blood count was: red cells, 5,156,000 per c.mm.; white cells, 9,900 of which 2.27% were eosinophiles; hemoglobin, 110%. Color index, 1.06. Blood Wassermann, negative. Rhamy and Metts[15] reported a group of these cases from the same county of Indiana.

Lamblia (Fig. 80).—This family of pear-shaped organisms is characterized by a deep concavity on the inferior surface and by 4 pairs of flagella,

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[15] Jour. of A. M. A., Apr. 15, 1916, lxvi, p. 1190.

3 on the edges of the concavity and 1 at the posterior extremity. The parasite found in man has been named: Lamblia intestinalis, Cercomonas intestinalis (Lambl), Cercomonas coli (May), Trichomonas intestinalis (Leuckart).

Its protoplasm is hyaline or very finely granular and never contains solid inclusions, it has a very fine cell-membrane and a nucleus which is dumb-bell shaped, situated at the base of the concavity. It has 4 pairs of flagella of almost equal lengths (9 to 14μ), 1 pair on each side of the concavity, 2 pairs at the projection on the inferior edge of the concavity and 1 pair at the end. The motile forms vary from 10 to 21μ in length and from 5 to 12μ in width. This parasite lives in the jejunum and duodenum, each fastened on the top of a columnar cell which it embraces with its concavity. In some cases they are present in such numbers that they form a membrane covering the mucosa. Those which reach the large intestine become encysted, taking the form of round or oval bodies from 10 to 14μ long and 8 to 10μ wide with a very distinct membrane surrounding the organism. The motile parasite is seldom seen in the stools unless the patient has a severe diarrhea in which case they are seen thrashing about rapidly and very aimlessly. The stools of a patient who has taken a large dose of Epsom salts should be examined as fresh as possible and on a warmed stage. The number in the stools may be enormous; even, it is estimated, 18,000,000 in 24 hours. They may be recognized by their concavity and their dumb-bell-shaped nucleus. Their most important hosts are the mouse, rat, rabbit, dog, sheep, cat, etc. Men are evidently infected from water. They have been found principally in children. Their pathogenicity is uncertain yet they may aid in producing the symptoms of other diseases and they certainly thrive best in patients with intestinal troubles.

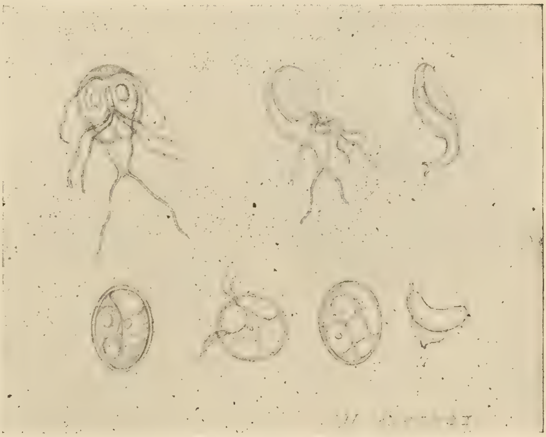

FIG. 80.—Lamblia intestinalis, showing the motile form in different positions, and stages of its encysting. × 900.

It is always interesting to watch this organism encyst itself. It first withdraws its tail flagella, then becomes more oval until the concavity finally disappears, the flagella for a while projecting from its edges. In some cases the markings of the encysted form, commonly taken to indicate the folds of the parasite, seemed to be the edges of this closed cavity. A membrane could in some be distinctly seen.

The *protomonadina*, or forms which have 1 or 2 equal flagella or 1 principal flagellum and 1 or 2 smaller ones, are much smaller than the above mentioned polymastigina. Two of the 3 forms occur in man: Cercomonadidæ, which have 1 flagellum and no undulating membrane, and the Trypanosomidæ which have 1 flagellum and an undulating membrane which reaches the whole length of the parasite.

Cercomonas Hominis.—These small flagellates, sometimes met with in the stools, are usually from 10 to 12μ, but vary from 8 to 16μ, in length. They are pear-shaped with 1 long flagellum even twice the body-length at the anterior end. Their motion is very rapid. Their pathogenicity is doubted. They have been found in other parts of the body also, including the sputum.

Infusoria.—The infusoria are bilaterally symmetrical protozoans which have a permanent shape, are ciliated, which contain contractile vacuoles and usually a macro- and micro-nucleus. The order which is of most importance to us now is that of Heterotricha, which are uniformly ciliated, but with a border of longer cilia around the peristome. Of these the most important is the Balantidium group.

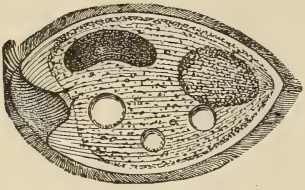
Fig. 81.—Balantidium coli. (Copied from Braun.)

Balantidium Coli or Paramæcium Coli.—Balantidium coli is an oval parasite (Fig. 81) from 60 to 100μ long and from 50 to 70μ broad uniformly covered with cilia. The mouth, at the anterior end, is a funnel or cleft-shaped entrance which extends ¼ the body length and which is surrounded by cilia about twice as long as those over the rest of the body. The ectosarc and the endosarc are clearly differentiated. The latter is finely granular and contains many fat or mucous droplets, starch granules, even red blood-corpuscles, leucocytes and bacteria. The nucleus is kidney- or bean-shaped and is accompanied by 1 or more accessory nuclei. Usually there are 2 contractile vacuoles which pulsate feebly. The surface is traversed by parallel longitudinal lines connecting the 2 poles and most distinct at the anterior end. The anal orifice is at its posterior end which is rather blunter than the anterior. This parasite lives especially in the colon but in severe cases may be found even in the jejunum. It may be present in the stools in such enormous numbers that 1 drop of blood-stained mucus may contain 200 organisms. The pathogenicity of these parasites has been doubted but it is now agreed that they may be the cause of a very severe and stubborn catarrh which may even be fatal. (Some regard them as secondary invaders in cases of intestinal catarrh, but others on the contrary believe that they can cause a catarrh which continues after they die out [Henschen].) Between 80 and 90 cases of apparently primary infection by these parasites are now on record, the most of them from Russia. A very good description of the condition is given by Strong and Musgrave.[16] Klimenko[17] believes that they first cause a diarrhea by mechanically irritating the rectal mucosa and later a catarrhal or even ulcerative colitis; that they invade the intestinal wall, enter the blood-vessels and sometimes cause emboli to distant organs, but that their action is chiefly mechanical is indicated by the absence of any degenerative or inflammatory changes which would point to the action of a toxin.

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[16] Johns Hopkins Hosp. Bull., February, 1901.

[17] Beitr. z. path. Anat. u. allg. Path., 1903, Bd. 33, p. 281.

Enthelmintha—Trichina Spiralis.—The adults of trichina spiralis have not as yet been found in the stools of man. They may be studied in the intestines of rats, pigs, dogs, and cats. The male worm is from 1.4 to 1.6 mm. long and 0.04 mm. wide; the female, 3 to 4 mm. long and 0.06 mm. wide. After the ingestion of infected meat the capsule of the encysted embryos is digested by the gastric juice and the liberated embryos mature rapidly in the small bowel. On the second day the males die and the females bore their way through the mucosa either of the villi or at the base of the Lieberkuhn glands and lie in the lymph spaces where they viviparously hatch their young (which are 0.09 to 0.1 mm. long, and 6μ wide) into the lymph and blood-stream. These travel passively in the blood stream, then bore their way into the tissues and in 9 or 10 days come to rest in the muscles. They are then about 1 mm. long. A capsule forms around them, which in about 1 year begins to calcify (see Fig. 83).

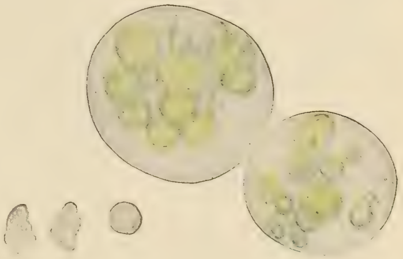

Fig. 82.—Ameba coli (Entameba dysenteriæ). An uncommon, very hyaline, and very ameboid form of parasites, usually filled with red blood-cells. The small forms are true ameba from a normal case. Entameba coli, drawn to the same scale. × 400.

Ascaris Lumbricoides.—Ascaris lumbricoides, the ordinary "round worm," is so common an intestinal parasite, that it is found in the stools of about 0.4% of all persons examined (Garrison, Ransom, and Stevenson). The female is from 20 to 40 cm. long, 5 mm. thick, and has a straight and conical tail. The male is from 15 to 25 cm. long, 3 mm. thick, and has a posterior end which is bent ventrally into a hook and which terminates in 2 spicules. The mouth of both sexes is surrounded by 3 papillæ. The color of these worms is gray or a dirty reddish-brown. While this worm lives as a rule in the small intestine, so is usually met with in the stools, yet, especially in cases with high fever as typhoid, it may become very actively migratory, make its way into the stomach and appear in the vomitus, or crawl up the esophagus and appear in the nose or enter the Eustachian tube or crawl down into the trachea. To obtain the worm itself a dose of santonin will have a therapeutic as well as a diagnostic value. Its fertilized eggs (Fig. 84, *d*, *e*), which often appear in the stools in large numbers, are elliptical, from 50 to 70μ long and 40 to 50μ wide. (Those which we have measured varied from 65 to 80 by 45 to 55μ) and have an unsegmented protoplasm surrounded by a thick transparent shell, which in turn is covered by a thick, gelatinous very lumpy envelope which usually is bile-stained.

The unfertilized ova appear so different from the fertilized eggs that for a long time they were not recognized as eggs at all. Dr. O. T. Logan,[18] who was one of the first to interpret them correctly, convinced us that the cell represented at the left hand edge of Figure 75 as a vegetable cell was certainly a typical unfertilized ascaris egg. Houghton (personal communication) in a series of fecal examinations of 500 patients in Wuhu, China, found that 71.2% were infected with ascaris. Of these 38.6% passed fertilized and unfertilized eggs, and 3% unfertilized eggs only.

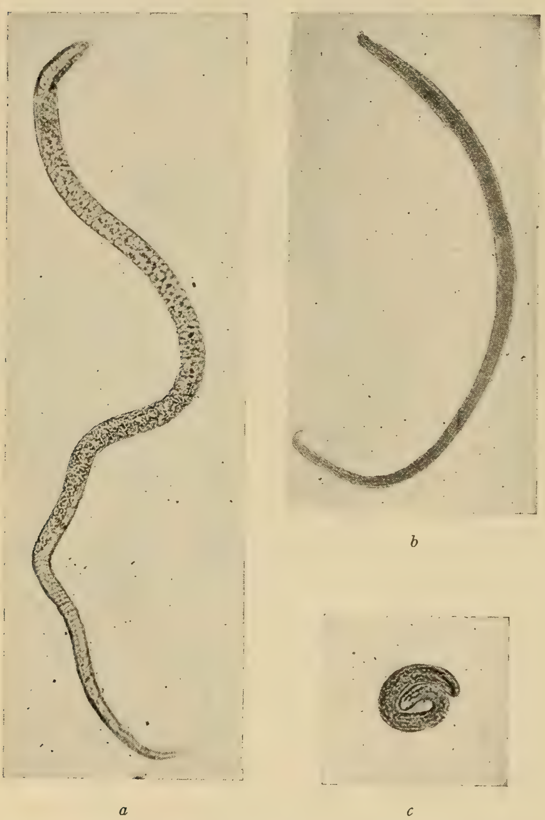

Fig. 83.—Trichina spiralis. *a*, adult female. *b*, adult male. × 90. *c*, embryo. × 400. (I am indebted to Dr. C. L. Overlander, of Boston, for these photographs.)

That the young worm from the recently hatched egg migrates through the intestinal wall and that some of these reach the lung and thence the bowel via the trachea and esophagus thus infecting the host has been demonstrated by Ransom.[19]

Oxyuris Vermicularis.—This little parasite (Fig. 86) occurs in the rectum and colon as high as the cecum where it inhabits the appendix. It may, however, travel even to the stomach and through the uterus and tube to Douglas's cul-de-sac. According to some it can bore its way through the intestinal wall and thus cause an abscess. About 0.8% of all adults examined are infected by this worm. These worms are white in color. The adult male is from 3 to 5 mm. long. Its posterior end is bent into a ventral hook. The female is 10 mm. long and 0.06 mm. wide. The eggs which are 50μ long and 16 to 20μ wide have a characteristic asymmetry. The

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[18] Rep. Am. Soc. Trop. Med., 1908.

[19] Jour. A. M. A., Oct. 18, 1919, vol. 73, p. 1210.

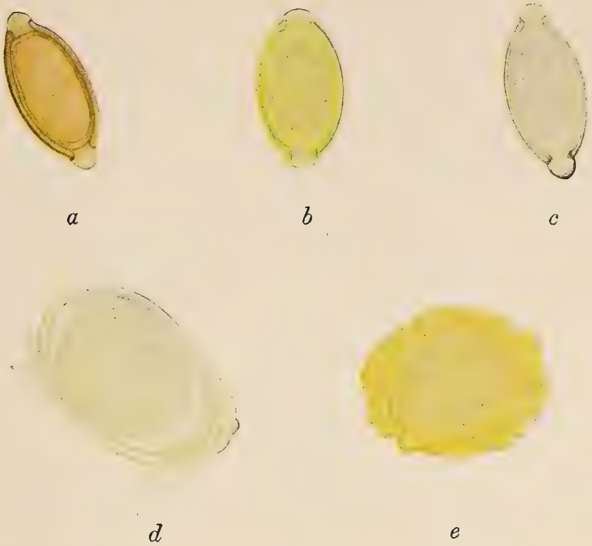

FIG. 84.—Parasite eggs in stools. *a*, *b*, *c*, eggs of trichocephalus dispar, showing the different colors (species?); ***d***, ***e***, ascaris lumbricoides; *d*, envelope lost; ***e***, perfect. × 400.

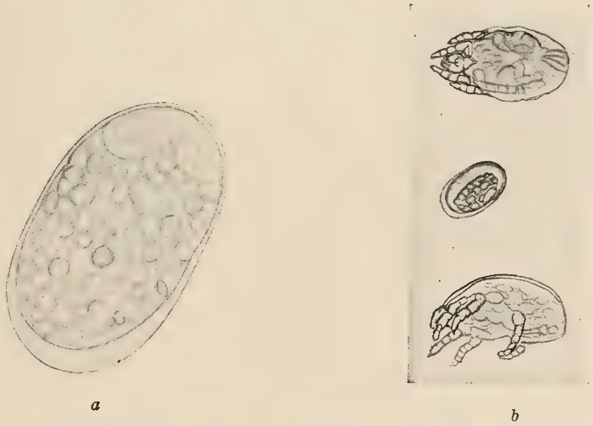

FIG. 85.—Eggs of Tyroglyphus siro (cheese- or flour-mite). *a*, an egg magnified × 400 to allow a comparison of size with the eggs of Fig. 84. *b*, in the center an egg and above and below two mites soon after they hatched and had developed somewhat. × 100. NOTE.—We picture these eggs merely as a warning to the student that not all the eggs he may find in the stools are eggs of important parasites. One not infrequently finds eggs of the great variety of harmless insects, etc., which are swallowed with the food. When in doubt concerning an egg it should be carefully measured and then the attempt made to hatch it. If still in doubt the specimen should be sent to the Washington Laboratories (Department of Health).

parasite leaves the rectum at night to lay its eggs on the skin surrounding the anus and it is then that the itching occurs. The eggs when deposited contain a well-developed embryo. The skin around the anus should be scraped both for the adults and eggs, for it is rare to find them in the stools, except in the mucus which the stool gains on passing through the anal canal.

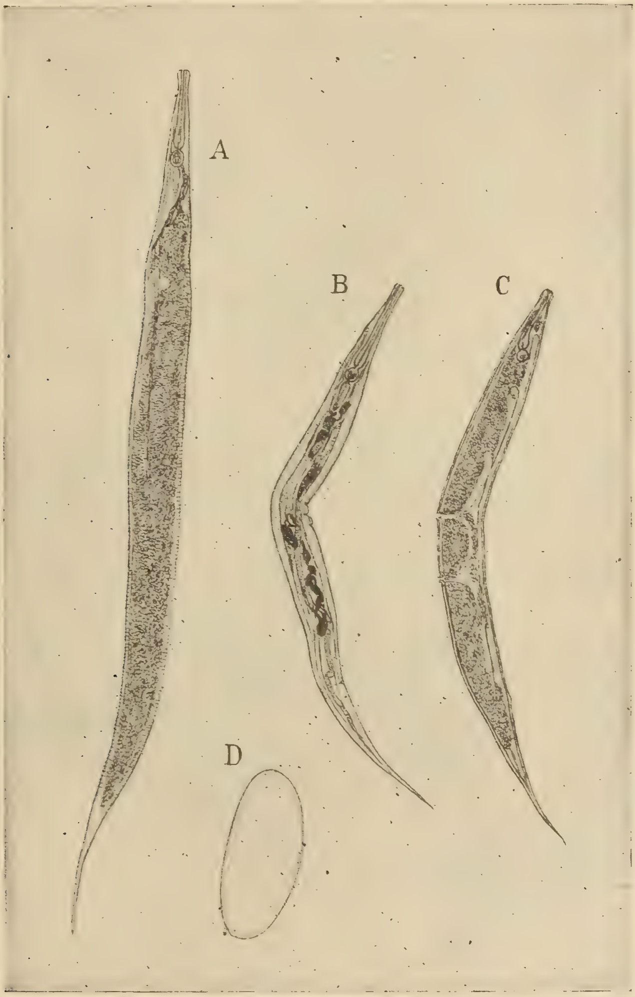

Fig. 86.—Oxyuris vermicularis. A, B, and C, adults; A and C are females full of eggs. × 12. D, egg, × 400.

Ankylostomum Duodenale and Uncinaria Americana.—These 2 varieties of hookworm (Figs. 87–93) which belong to the nematode family Strongyloidæ cause some of our severest anemias. They live in the duodenum, jejunum and ileum, sometimes thousands in 1 person but seldom more than a few hundred. They do not multiply in the bowel but the individual worms may live there for 5 years. They have recently attained great importance in this country through the demonstration by Stiles that they are the common cause of the "anemia of the South." In 500 cases chosen at random they were present in 3% of the individuals.[20] Stiles showed that there are in reality 2 varieties of hookworm in the Southern States, the form described years ago in Europe and another form to which he gave the name Uncinaria Americana. They have long been known as the cause of bricklayer's anemia, tunnel-workers' anemia, Egyptian chlorosis, miners' anemia, etc. The best description of these parasites is that given by Stiles.[21]

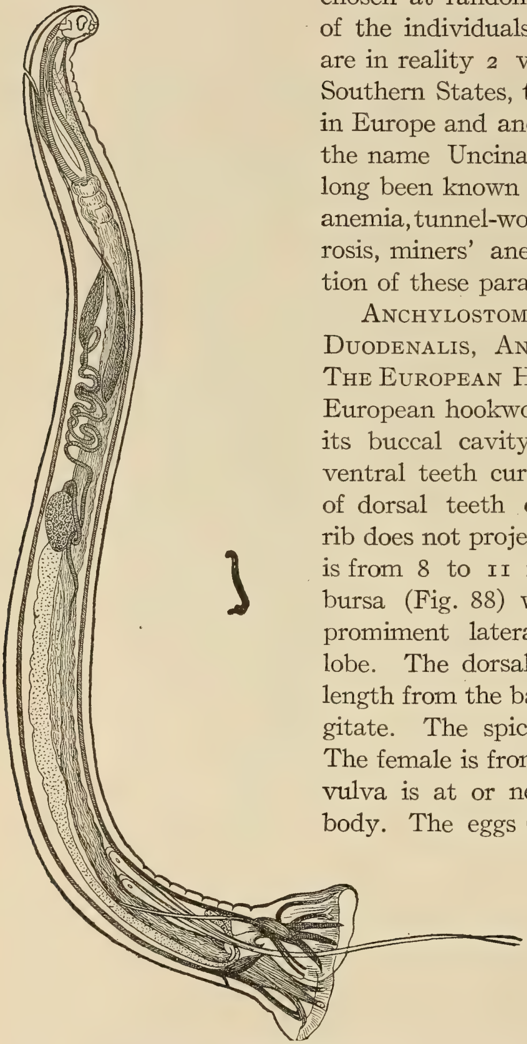

FIG. 87.—Ankylostomum duodenale, natural size to right, much magnified male on left. (From Braun.)

ANCHYLOSTOMA DUODENALE, UNCINARIA DUODENALIS, ANKYLOSTOMUM DUODENALE, THE EUROPEAN HOOKWORM.—The body of the European hookworm is cylindrical (Fig. 87), its buccal cavity (Fig. 91) has 2 pairs of ventral teeth curved like a hook and 1 pair of dorsal teeth directed forward; the dorsal rib does not project into the cavity. The male is from 8 to 11 mm. long. It has a caudal bursa (Fig. 88) with dorso-median lobe and promiment lateral lobes united by a ventral lobe. The dorsal ray divides at a point ⅔ its length from the base, each branch being tridigitate. The spicules are long and slender. The female is from 10 to 18 mm. long. The vulva is at or near the posterior third of the body. The eggs (Fig. 89) are ellipsoid, 52 by 33μ, and segmented when laid. Development is direct without intermediate host.

NECATOR AMERICANUS, UNCINARIA AMERICANA (Stiles, 1902).—The American form of hookworm differs from the European in that its buccal cavity (Fig. 90) has a dorsal pair of prominent semilunar plates or lips and a ventral pair of slightly developed lips of the same nature, but no hook-like teeth. The dorsal conical median tooth projects prominently into the buccal cavity. The male is from 6 to 9 mm. long. Its

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[20] See also Smith, Am. Jour. Med. Sci., 1903, vol. cxxvi.

[21] Eighteenth Annual Report of the Bureau of Animal Industry, 1901.

caudal bursa (Fig. 92) has a short dorso-median lobe, which often appears as if divided into 2 lobes and prominent lateral lobes united laterally by an indistinct ventral lobe. The common base of the dorsal and dorsolateral rays is very short. The dorsal ray is divided to its base, its 2 branches being prominetly divergent and their tips bipartite. The spicules are long and slender. The female is 9 to 11 mm. long, the vulva in the anterior half of the body but near the equator. The eggs are ellipsoid, 64 to 72μ long by 36 to 40μ broad, some are segmented in utero and others contain a fully developed embryo when laid (Fig. 93).

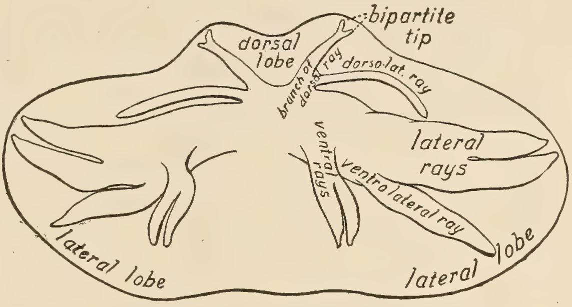

Fig. 88.—Caudal bursa of Uncinaria americana. (Schematic.)

The hookworm's eggs (Fig. 89) found in the stools may be unsegmented but the majority have already divided into 4, 8, 16, etc. segments. They have a thin, clear shell. The older the feces and the warmer the weather the more advanced will their segmentation be. This is especially true of Uncinaria Americana.

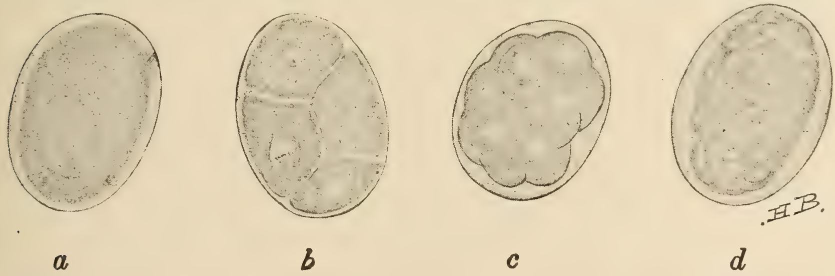

Fig. 89.—Eggs of Uncinaria duodenalis. *a*, unsegmented; *b*, with four segments and showing nuclear spindles; *c* and *d*, later stages of segmentation. × 400.

To find uncinaria eggs it may be sufficient to mix a small particle of stool with a drop of water and spread it on a slide, but if they are not numerous it is better to follow the various suggestions of Pepper.[22] That is, to dilute the stools with about 10 volumes of water, strain it through 2 or 3 layers of gauze in a funnel, and then centrifugalize it until the sediment is just thrown down. The supernatant fluid is now poured off, more water added, the tube is well shaken, the stool is again centrifugalized and this sediment examined. Again, use is made of the tendency of uncinaria eggs to stick to glass. A drop of the sediment is put on a glass slide and the slide is gently immersed in water. This will wash off much of the sediment while the uncinaria eggs will stick. Another drop of the sediment is then added to the same spot and the slide again immersed. The process is repeated several times. In this way one may obtain fields which abound in eggs. One disadvantage of this method is that the eggs of other parasites will be lost.

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[22] The Jour. of Med. Research, March 1908, vol. xviii, No. 1, p. 75.

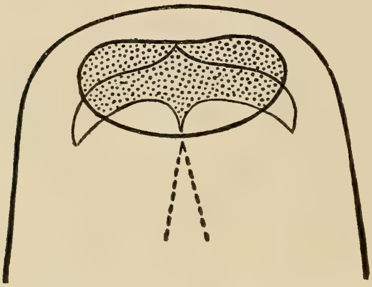

FIG. 90.—Head of Uncinaria americana. (Schematic.)

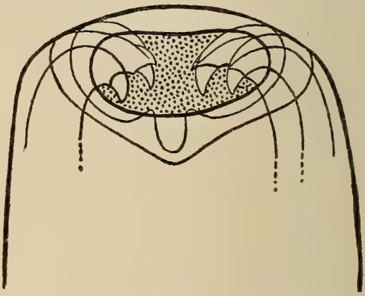

FIG. 91.—Head of Uncinaria duodenalis. (Schematic.)

The adults may be found in the sedimented stool following a small dose of thymol and then of oil. They are usually red from the blood which fills their intestine.

TRICHOCEPHALUS TRICHIURIS—TRICHOCEPHALUS DISPAR—TRICHOCEPHALUS TRICHURIA—WHIPWORM.—The ordinary whipworm is from 4 to 5 cm. long, ⅗ of its length consisting of a whip-like tail. It inhabits the cecum, but also the colon and rarely the small intestine. This is, perhaps, the most common of intestinal parasites since about 10.3% of adults in this country are infected, but 45% in some parts of Germany and even 100% in Southern Italy. The eggs (Fig. 73) are very characteristic, from 50 to 54μ long and 23μ wide, with an unsegmented yolk and a very thick shell, into each pole of which is inserted a plug. We have found eggs which were certainly those of this worm which had no plugs. These eggs present an interesting variety of colors, some being light lemon-yellow, some deep yellow and some a dark brown. While harmless as a rule, this worm may cause enteritis and even a very severe, even fatal, anemia. Becker[23] classifies the symp-

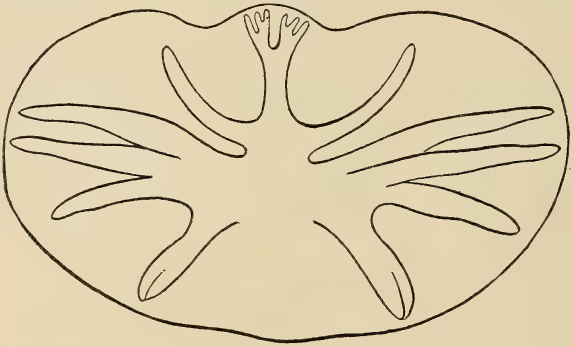

FIG. 92.—Caudal bursa of Uncinaria duodenalis. (Schematic.)

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[23] Deutsch. med. Wochenschr., June 26, 1902.

toms of this infection as follows: gastro-intestinal symptoms among which are diarrhea due to ulcers or catarrh, blood in the stools and pain even simulating appendicitis; nervous, some symptoms simulating meningitis (beriberi even has been blamed to this worm) and anemia, with all its symptoms

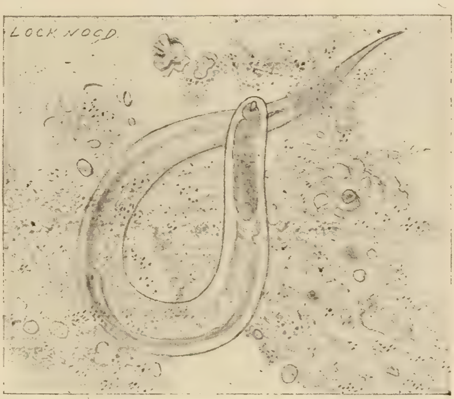

FIG. 93.—Embryo of Uncinaria (americana?) found in the stool. X 400.

STRONGYLOIDES STERCORALIS; ANGUILLULA STERCORALIS ET INTESTINALIS; LEPTODERA STERCORALIS ET INTESTINAL—RHABDITIS STERCORALIS—RHABDOMENA STRONGYLOIDES, ETC.—The rhabditiform larvæ of strongyloides stercoralis found in the stools measure from 0.3 to 0.6 mm. long and from 16 to 22μ wide. Since these embryos are very active in their motion the best way to find them is to make a depression in the fecal mass, fill it with water, then place the stool in a thermostat and the next day examine a drop of this water for these eel-like active worms. The eggs are very rarely present in the stools since practically all hatch in the bowel where all stages of the embryos may be found. If found, they could hardly be distinguished from those of Uncinaria duodenalis although they are perhaps a little larger (measuring from 65 to 70μ long and from 34 to 39μ wide) and would contain a further developed embryo.

The adult female resembles a filaria. It measures from 2.1 to 2.2 mm long and 32 to 39μ wide. The body increases slightly and gradually in size from the head to the posterior quarter, and then terminates rather suddenly in a short tail. The male is about ⅓ smaller. The worms are abundant in the duodenum and scanty in the jejunum. The adults are very rarely found in the stools. This infection was present in about 0.6% of a series of patients examined in Washington. Houghton, writing from Wuhu, China, states that from 0.9 to 1% of all patients examined there harbored this parasite, but he emphasized the local distribution of the parasite by saying that this worm has not yet been reported north of the Yangtse River, nor in Manchuria and Korea.

TREMATODES.—Infections in the Far East by Schistosoma Japonicum have recently been carefully studied by Katsurada in Japan, by Catto in Singapore, by Woolley in the Philippine Islands and by Houghton[24] in China (in the provinces of Hunan, Honan, Hupeh, Kiangsi and Anhui).

Houghton found that 8% of all male patients admitted during 1 year to the Wuhu General Hospital, Anhui, harbored this worm. Almost all of these were farmers and boatmen from the southern half of the province of Anhui and within a radius of 100 miles of Wuhu. The area of distribution of this worm is sharply defined since Houghton writes (personal communication) that in the province adjoining Anhui on the North the worm has not yet been found. The distribution of the parasite in China follows in general the flat, low-lying lands of the central valley of the Yangtse and the valleys of tributary waters. In Anhui at least its presence is limited to the rice-growing divisions of the country. No cases from the hill or mountain districts have appeared. Peake [25] reports that it is common among raftsmen in Hunan. In some localities it is claimed that 1 in every 3 or 4 or more of the farmers and boatmen shows the physical signs of this infection.

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[24] Trans. of the Society of Trop. Med. and Hygiene, June, 1910, vol. iii, No. 7, p. 342.

Well-marked cases of this infection have enlarged liver and spleen, cachexia, eosinophilia, ascites, greatly exaggerated knee-jerks and bloody stools. The percentage of eosinophile cells in the blood varies from 10 to 51% (average of 25%) of a total leucocyte count of from 2000 to 8500 per c.mm. The anemia is seldom marked (Hb averages 80%). Less well-marked cases may have the enlarged spleen and the eosinophilia or the eosinophilia alone. Very few have ova in the stools as the only symptom.

The ova may be found in the blood, but are more easily demonstrated in the stools although it is not always easy to find them there, since their envelopes are sticky and so gather debris in the stool and leucocytes in the blood. They closely resemble the ova of Ascaris lumbricoides (they measure from 60 to 90μ in length and 30 to 50μ in width), for which, under the low power, they may easily be mistaken, although they are more refractile, with envelopes not as deeply bile-stained and bosses not as prominent. These ova have a yellowish-brown color, are oval and have neither operculum nor spine. In the fresh stool the embryo, shaped like a melon seed, is quiescent in the egg but a little later the cilia are seen to be in motion. The free-swimming miradidium is found only after the stool has stood for about 10 hours. It can be kept alive in water for at least 5 days.

The adult male measures about 10 mm. in length and 0.5 mm. in breadth. The slender, almost cylindrical female, is from 8 to 12 mm. long and 0.113 mm. in diameter. The skin of this worm, unlike that of Schistosoma hematobium, is smooth. The adult worms are found in the smaller mesenteric blood-vessels (perhaps in the arteries especially). The ova are found in necrotic areas in the mucosa and submucosa of both the small and the large bowel, but some are found in the subperitoneal tissue.

Fasciolopsis buski (Distomum buski, D. crassum) the largest of the trematode parasites of man, measures from 34 to 70 mm. in length and from 5.5 to 15 mm. in width. Its eggs are from 120 to 130μ long and from 77 to 80μ wide. They have a thin shell, a very small operculum, and granular contents. Only a few cases, all of them intestinal infection, have been reported, and these were in the Far East. These patients suffered from a moderate diarrhea which continued for years, emaciation and anemia. The 1 parasite described under the name of Distomum rathouisi was, according to those in a position to judge, probably a specimen of Fasciolopsis buski.[26]

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[25] China Medical Journal, 1908.

Distomum Lanceolatum.—The body of Distomum lanceolatum is pointed at both ends and measures from 8 to 10 mm. long and from 1.5 to 2.5 mm. wide (see Fig. 94). The eggs, which are yellowish when fresh and dark brown later have thick shells and measure from 38 to 45μ in length and from 22 to 30μ in width. They contain an oval miracidium of which the anterior part alone is ciliated, and which hatches only in the intestine of some intermediary host, perhaps of a slug. This lancet fluke is a relatively rare parasite of the biliary ducts of the European and American domestic animals. Thus far it has been found but seven times in man.

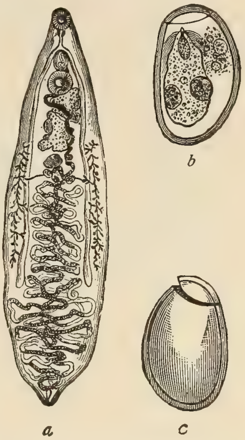

Fig. 94.—Dicrocelium lanceolatum, *a*, adult; *b*, egg with embryo; *c*, empty shell (From v. Jaksch.)

Fasciola Hepatica.—The liver fluke is a widelyspread parasite inhabiting the bile-ducts of many herbivorous mammals. The adult measures from 20 to 30 mm. in length and from 8 to 13 mm. in breadth and has a definite head cone. The ova are yellowish brown in color, oval, measure from 130 to 145μ long and from 70 to 90μ wide and have a cap-like lid. The elongated miracidium which is completely ciliated, escapes from the egg after this has been in the water for a few weeks and swims free until it enters a water-snail in which host it passes through the stages of sporocyst, redia and cerecaria. The cerecaria become encysted on the grass of the meadows and is eaten by sheep, cattle, etc. Only 23 cases have been reported in man.

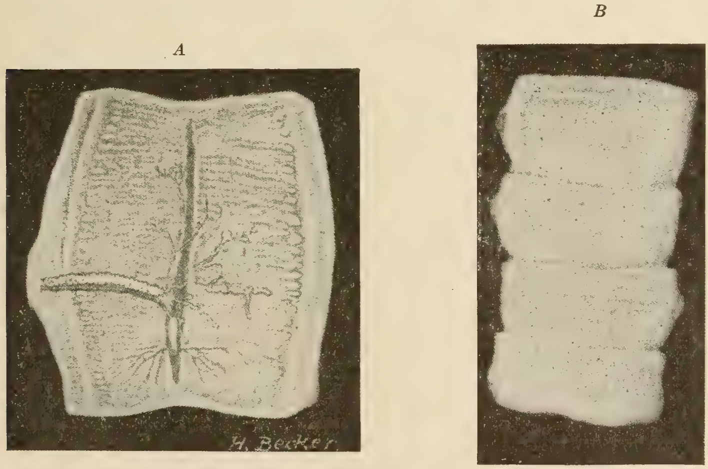

Fig. 95.—*A*, ripe link of Tenia saginata. × 3. *B*, four unripe links. × 3.

Cestodes.—In a suspected case of tapeworm infection it is always important that segments be found before the treatment is undertaken.

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[26] Jeffreys and Maxwell, Diseases of China, 1910.

Mucus casts of the intestine, certain food constituents, etc., are often mistaken for tapeworms. The treatment can be termed successful only if the head can be found (to find this, the stool is well mixed with water and allowed to settle for about 10 minutes, the heavy head will settle to the bottom, and the fluid is then decanted and this procedure repeated several times) or 3 months have passed with out the reappearance of segments.

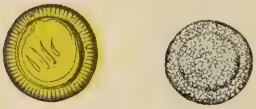

FIG. 96.—Eggs of Tænia saginata. × 400.

TÆNIA SOLIUM (Figs. 97 and 98).—The infection with Tænia solium is derived from Cysticercus cellulosæ of pork. The adult worms in the intestine average about 3m.long, although much longer have been described. The head varies from 0.6 to 1 mm. in diameter and has four suckers from 0.4 to 0.5 mm. in diameter and a rostellum with a double crown of 22 to 32 hooks from 0.11 to 0.18 mm. long. The neck is about 3 cm. long, and is unsegmented. The ripe segments are from 9 to 10 mm. long by from 4 to 5 mm. wide. The genital openings are marginal and alternate in a fairly regular manner. The shape of the uterus is one of the most characteristic points for identification. It consists of a large median stem with from 7 to 10 coarse branches on each side, each of which branches dendritically. The eggs are round or oval, about 35μ in diameter and the shell very thin and surrounded by a thick embryonic shell, radially marked and often yellow in color. This worm is very rare in America. The only specimen of Tænia solium which we have seen (Figs. 97 and 98) was discovered in Baltimore by Dr. Thos. Boggs. The most of those exhibited in museums are wrongly labelled.

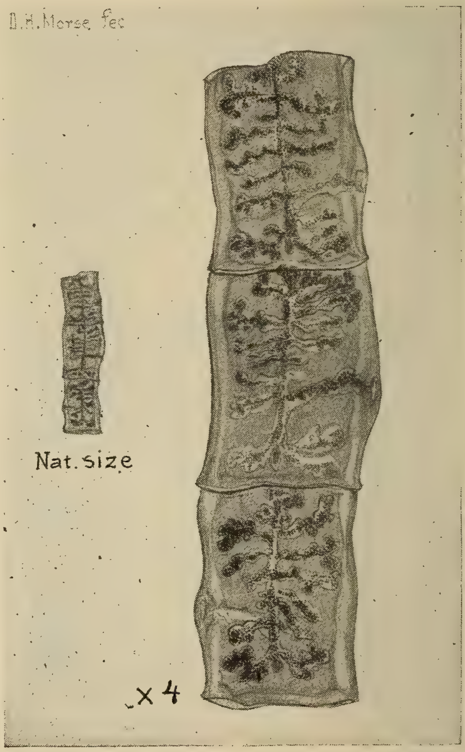

FIG. 97.—Tenia solium. Mature links from a case recently discovered in the Johns Hopkins Out-patient Department. (Kindness of Dr. T. R. Boggs.)

TÆNIA SAGINATA.—The beef tapeworm, which infection is derived from the cysticercus of beef and, perhaps, of sheep, is quite common in this country. The adult worm varies from 4 to 8 m. or more in length. The head (Fig. 99) is from 1.5 to 2 mm. in diameter, is cuboid in shape and has 4 suckers, each 0.8 mm. in diameter and no hooks. The neck is long and delicate. The ripe segments (Fig. 95) are from 16 to 20 mm. long and from 5 to 7 mm. wide. The over-ripe segments are longer and somewhat more slender. The genital openings are marginal and alternate irregularly. The uterus is characterized by the great number of its fine branches, from 20 to 35 on each side of the median stem each of which branches dichotomously. The eggs (Fig. 96) are spherical or oval, from 30 to 40μ long and from 20 to 30μ wide and have a thin shell surrounded by an embryonic shell which is thick and radially striated.

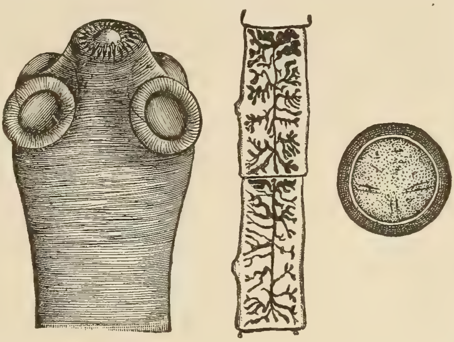

Fig. 98.—Tænia solium: Head (magnified), proglottis (actual size), and egg (magnified). (Zeiss's eye-piece IV, objective IV.) (From a preparation by Cori and v. Jaksch.)

Hymenolepsis Nana—Tænia Nana (Fig. 100).—This dwarf tapeworm is not at all uncommon in man, as Stiles[27] has shown, who found it in 16 of 3500 persons examined.

The worm (Fig. 90) is from 10 to 15 mm. long and from 0.5 to 0.7 mm. broad. Its spherical head, which is from 0.25 to 0.3 mm. in diameter, has 4 suckers and a row of 24 to 30 very small and characteristically shaped hooks (14 to 18μ) long. The segments, about 150 in number are short (0.014 to 0.030 mm.) and relatively broad (0.4 to 0.9 mm.)

The egg is characteristic in appearance. It is spherical or oval in shape from 30 to 37 by 48μ in its 2 diameters and has 2 distinct thick membranes, the inner of which has at each pole a more or less conspicuous process with filamentous appendages.

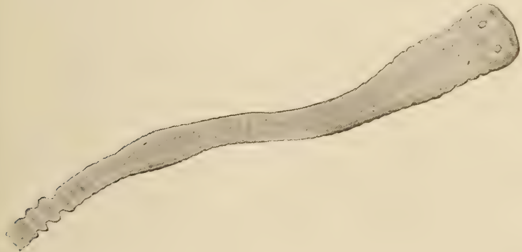

Fig. 99.—Head of Tænia saginata. × 5.

The parasite inhabits the ileum where a few or many thousands may be present. It is probably the same as the very common form in rats.

Dipylidium Caninum—Tænia Cucumerina. — This tapeworm is from 15 to 35 cm. long and from 1.5 to 3 mm. broad. Its head is club-shaped with a rostellum and 3 or 4 rings of hooklets. The eggs are circular, from 43 to 50μ in diameter and the shell thin. It occurs in dogs, cats, and rarely in man, and then especially in children.

Bothriocephalus Latus.—This tapeworm (Fig. 101), the largest parasite of man, is exceedingly common in the maritime countries of Europe, in Ireland and in Japan. A rapidly increasing number of cases is being discovered in America. The cysticercus stage occurs in fish. It often reaches 8 m. in length and a few even 50 feet. The infections are often multiple. In Willson's case[28] (this is 1 of the best reports) 2 worms were

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[27] New York Med. Jour., 1903, vol. lxxviii, p. 877.

[28] Am. Jour. of Med. Sci., 1902, vol. cxxiv.

present whose total length aggregate 82 feet. One man may harbor from 50 to 100 of these worms but in such cases the individual worms are much shorter, perhaps from 3 to 5 feet long. The head is 1 mm. broad, from 2 to 3 mm. long, is flat, almond or spoon-shaped, with 2 deep grooves at its sides which serve as suckers. It has very little neck. The ripe segments, which begin about 50 c.c. from the head, increase in size until they reach from 10 to 15 mm. broad and 3 to 4 mm. long. The genital opening is on the side, not the edge, and around it the uterus radiates in the form of a rosette. The distribution of these organs is more regular than that of the septa of the segments. Willson considers that the presence of these imperfect or abortive segments is very characteristic of this family of worms. The eggs are characteristic. Their diameters are 70 and 45μ, their shell thin, the contents granular, giving them a mulberry-like appearance. The shell has a lid which may be open or closed. In very young eggs the lid is not evident. It may not be in older ones but can be rendered visible by pressure on the glass. The eggs are important in diagnosis since the segments rarely appear in the stools, although when theydo it is in great numbers. This tapeworm is important since some, a small percentage of its hosts, develop an anemia which hematologically cannot be distinguished from primary pernicious anemia, and these recover rapidly after the worm has been expelled. Dehio thinks the worm, to produce this effect, must either die or at least be diseased and so furnishes a toxin which affects the bone marrow.

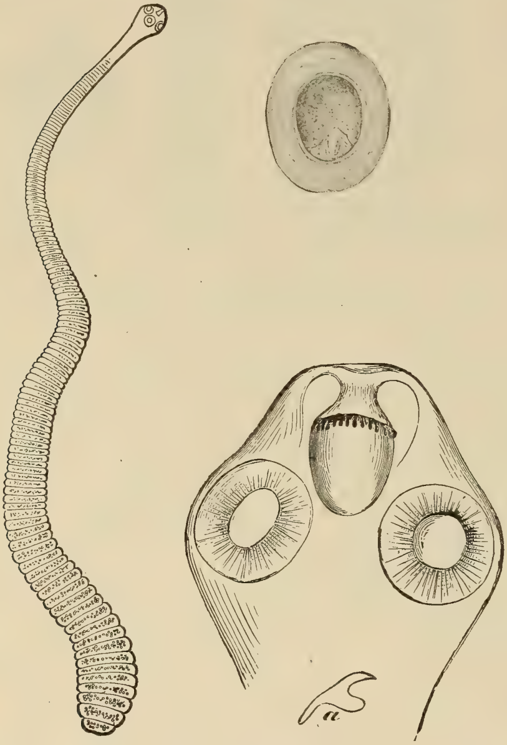

FIG. 100.—Hymenolepis nana. Adult (left), head (right), egg (above); *a*, hooklet. (From Braun.)

The eggs of Schistosoma Hematobium (see page 418) also are met with in the stools of infected cases (Figs. 102 and 103).

Plant Parasites.—*Yeasts* are often present in normal stools. Moulds are rare. *Blastomycetes* are found in the stools of patients with systemic infection with this parasite.[29] *Oidium albicans* has very rarely been found in the stools of children. *Sarcinæ* are often found in cases with dilated stomach and when present in large numbers may cause a diarrhea by the products of their fermentation.

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[29] Fontaine, Hasse and Mitchell, Arch. Int. Med., Aug., 1909.

Bacteria.—The bodies of bacteria form a considerable part of the mass of the stools (see page 387) but the vast majority of these organisms are dead. Almost any organism may gain entrance accidentally to the intestine, but there is a flora of bacteria so constant in the bowel that its presence may be considered normal. Among these are: Bacillus coli communis (see page 286), Bacillus lactis aërogenes (see page 288), and for the suckling, Bacillus bifidus.

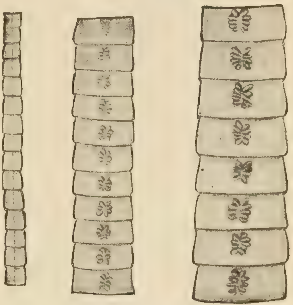

Fig. 101.—Bothriocephalus latus. (From Braun.)

Bacillus Bifidus [30] (Teissier, Paris Thesis, 1900).—This organism (see Fig. 104) would appear to be a normal inhabitant of the intestines of the suckling and to disappear soon after the child is weaned. When, however, it persists, its presence would seem to be associated with symptoms of intestinal intoxication. It is an organism from 2 to 4μ long, and often in pairs. Its most characteristic shape is that of the letter Y. Involution forms are common. Its great interest is that this is 1 of the few intestinal organisms which are not decolorized by Gram's method. It is a strict anerobe. Among other important organisms are: Bacillus alkaligenes (see page 288) and the proteus group (see page 288). Among the important pathogenic organisms which sometimes, even frequently, are found in the stools are: Bacillus pyocyaneous (see page 289); Bacillus aërogenes capsulatus (see page 289); Bacillus tetani (see page 290), the Staphylococcus group (see page 19) and the Streptococcus group (see page 19). A great many thermophilic and acidophilic organisms also are present which will not grow on ordinary media or at ordinary temperatures. The thermophilic organisms grow only at temperatures above 40° C. and some of them best at 60° C. They, therefore, cannot multiply in the intestines and those found in the stools must have been swallowed with the food. The present opinion is that the lower bowel at least is not a favorable habitat for organisms and that, as a result, the majority of those present in the stools are dead.

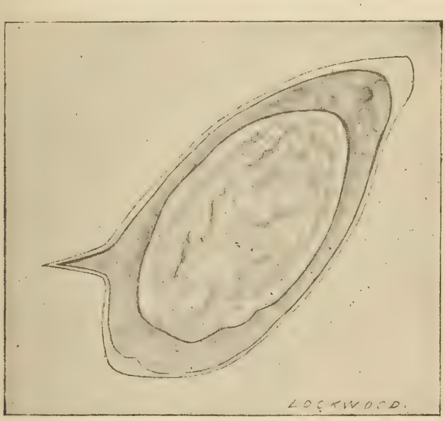

Fig. 102.—Egg of Schistosoma hæmatobium found in stool. Embryo dead. × 400.

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[30] Teissier, Paris Thesis, 1900.

Tubercle Bacilli.—In the search for Bacillus tuberculosis it is useless to try to digest a solid stool. Mucous masses, if present, should be selected, especially those which are blood-stained or purulent, and these are treated as is sputum. Indeed the diagnosis of pulmonary tuberculosis has frequently been made in this way, especially in children; but this is a rather remote possibility in the case of a careful adult. In intestinal tuberculosis these organisms are often present in abundance and yet in many such cases none at all are found, which has led to the supposition that they have been destroyed.

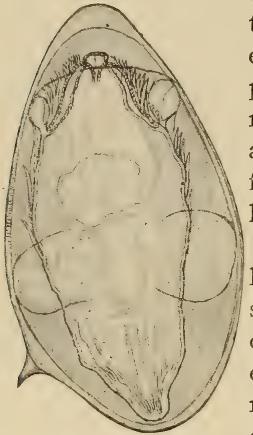

Fig. 103.—Egg of Schistosoma hæmatobium found in stool. Embryo alive. × 400.

Page's method of searching for Bacillus tuberculosis in the solid stool is to suspend a piece half the size of a pea in 1.5 c.c. of distilled water, add 54 c.c. of a mixture of equal parts of absolute alcohol and ether and centrifugalize this for 10 minutes; a smear made of the sediment is fixed to the slide with a drop of egg albumin and stained as usual.

To isolate tubercle bacilli from the feces[31] the specimen is diluted with about 3 volumes of water, well mixed and then filtered through several thicknesses of gauze to remove solid food particles. The filtrate is saturated with NaCl and left undisturbed for 30 minutes. The floating film (which contains all the bacteria) is then collected with a deflagration spoon in a wide-mouthed bottle and an equal volume of *N* NaOH added. This is shaken well and left for digestion in the incubator at 37° C. for 3 hours during which time it is shaken every half hour. It is then neutralized to sterile litmus paper, with *N* HCl, centrifugalized and the sediment inoculated into several test tubes of Petroff's medium.

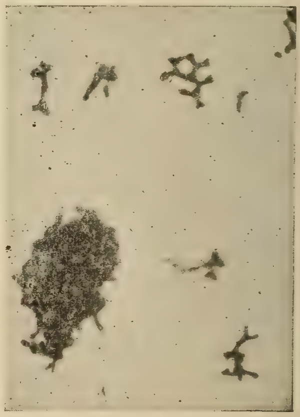

Fig. 104.—Bacillus bifidus. (Photomicrograph by Dr. Thomas M. Wright.)

The growth appears in from 2 to 3 weeks, that is, much more slowly than in sputum cultures, probably since these bacilli have become weakened during their passage through the bowel. Many probably die which may explain the negative results in clearly tuberculous cases.

Stools in Disease.—In typhoid fever the stool described as characteristic resembles " pea soup " in appearance, is copious, watery, of foul odor, alkaline in reaction and contains many triple phosphate crystals. Nevertheless in clinics which limit these patients to a careful diet such stools are rare and diarrhea is less common than is constipation. The stool is frequently blood-tinged, this tinging sometimes warning us of a coming hemorrhage. Pus (microscopic) is rare except in severe cases with extensive ulceration.

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[31] Petroff, Jour. Exp. Med., 1915, xxli, 41.

Bacillus Typhosus.—Many methods have been proposed for growing Bacillus typhosus from the stools.[32]

Drigalski and Conradi's medium [33] is the best.

Three pounds of minced beef are mixed with 2 liters of water and allowed to stand over night. The beef is then pressed, the beef juice is boiled for 1 hour, filtered and to this filtrate are added 20 gms. of peptone (Witte), 20 gms. of nutrose and 10 gms. of sodium chloride. It is then boiled again for 1 hour and filtered. To this filtrate are added 60 gms. of the best agar. It is then boiled for 3 hours (1 of which is in the autoclave). The fluid is made faintly alkaline to litmus, filtered and then boiled for ½ hour. While hot the litmus solution is added. The litmus solution is made by boiling 260 c.c. of litmus solution for 10 minutes, adding 30 gms. of the purest lactose and boiling again for 15 minutes. This litmus-lactose solution is now added while boiling to the hot agar solution described above. The mixture is then well shaken and then made very faintly alkaline.

One then adds 4 c.c. of a hot sterile 10 % solution of soda and 20 c.c. of a freshly prepared 0.1 % solution of Krystallviolett B., Höchst, in warm sterile distilled water. The medium is poured at once into large Petri dishes or kept in 200 c.c. flasks.

The resulting medium is a beef juice-nutrose-agar-lactose-litmus solution which contains 0.01 p. M. per mille, *i.e.*, per 1000 Krystallviolett. It hardens to a very firm mass, firm enough to prevent much diffusion of any acid formed. It will not become dry. The lactose is split by Bacillus coli, not by Bacillus typhosus. The colonies of colon bacilli will therefore turn the medium red and the typhoid blue (since this organism splips off basic bodies from the proteids). Krystallviolett will inhibit the growth of many other organisms, especially acid-producing cocci.

These authors recommend that several series of plates be inoculated in order to get the largest possible number of isolated colonies on a plate. If the stool is fluid 1 series of plates is inoculated with the undiluted stool and another with the stool diluted with 10 to 20 volumes of sterile normal salt solution. If the stool is solid it should be rubbed up to a homogeneous mass with sterile salt solution and various dilutions of this are used.

The stool is rubbed onto the surface of the medium. After inoculation the plates are left open for at least half an hour to allow the surface to dry, otherwise the colonies will coalesce. (The Krystallviolett will kill any air contaminations). When dry the plates are put into a thermostat at 37° C. and are examined in from 14 to 24 hours. The colon colonies will vary from 2 to 6 or more millimeters in diameter, are opaque, and will present a great variety of shades of red in color. The paratyphoid colonies are sometimes red and sometimes blue (see page 287).

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[32] For a critical review of this subject, see Pratt, Boston, M. & S. Jour., 1907, vol. 516.

[33] Zeitschr. f. Hyg. u. inf. Krank, 1902, vol. xxxix, p. 283.

The typhoid colonies have a diameter of from 1 to 3 mm., are blue or violet in color, glassy, not doubly refractile and are seldom opaque.

The colonies of the Bacillus subtilis group also are blue, but are so much larger than the typhoid that error is seldom possible. In some fetid stools the blue colonies of Proteus, Bacillus fluorescens and Bacillus fecalis alkaligenes may deceive. They are rare and can be distinguished by the agglutination test.

Peabody and Pratt [34] have shown the value of Malachite-green bouillon as an enriching medium. (The beef bouillon they used contained 1 : 1000 malachite green and had an acidity of 0.5% to phenolphthalein. The amount of dye and the acidity must be determined for each preparation of the malachite green used.) This will completely inhibit the growth of Bacillus coli, but while Bacillus typhosus often will grow luxuriantly in it, yet the dye does exercise some restraint over this organism also. Tubes containing from 10 to 15 c.c. of this medium are inoculated with 1 drop of the fluid stool or suspension of the stool and are left in the thermostat for from 10 to 24 hours. Then 1 drop of the culture is rubbed over the surface of a Drigalski-Conradi plate.

Drigalski and Conradi were able to grow typhoid bacilli from the stools of every case of typhoid fever they examined. Pratt and Peabody [35] were able to find it in but 21% of their cases. These bacilli were most numerous in those stools which contained blood. Many believe that while it is possible that all patients with typhoid fever may have a few living typhoid bacilli in their stools yet that the great majority are destroyed in the intestine and that only a few are alive when the stool reaches the rectum.

In severe ASIATIC CHOLERA the rice-water stools are quite characteristic. They are copious and consist chiefly of water secreted by the intestinal wall in which float small gray flecks which are masses of epithelial cells, cholera spirillæ and fat droplets. They have no fecal odor, are alkaline, almost acholic. sometimes are blood-stained and contain little albumin and much salt.

SPIRILLUM CHOLERÆ ASIATICÆ.—The spirillum of Asiatic cholera, or the "comma bacillus," is a small curved "comma-shaped" bacillus, about 2μ long and 0.5μ thick. It is very actively motile. It has a single, long, delicate flagellum at one end, it does not produce spores, it is readily stained in all bacterial stains and it decolorizes by Gram's method. Involution forms are common. This organism is very aërobic, a very rapid grower at room temperature on all ordinary media and also in some which are so poor in nutriment that other organisms cannot multiply at all in them. It will not grow on potato at room temperature, but will if in a thermostat. Its growth on gelatin, which it liquefies, is fairly characteristic in that the colonies soon appear as minute white points which resemble fragments of broken ground glass with granular irregular margins. Later, liquefaction begins and the colony sinks in the little cup of liquid cloudy gelatin which forms a halo around it. This organism produces much indol. It is very sensitive to acids.

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[34] Boston Med. and Surg. Jour., Feb. 13, 1908.

[35] Jour. Am. Med. Assn., 1907, xlix, 846.

The non-pathogenic spirilla are very common. More than 60 species with similar morphology, but different cultural characteristics, have been found in various drinking waters (*e.g.*, Spirillum Schuylkiliensis), while there is 1 in the mouth which will not grow at all in ordinary media.

Many pathogenic forms also have been described: Metchnikoff's spirillum, found in an epidemic in chickens, is pathogenic for birds while the true cholera spirillum is not, and is a more rapid grower than is the latter; Massea's spirillum is very pathogenic to pigeons and has 4 or 5 flagella; Finkler and Prior's form, from a case of cholera nostras, will grow as a dirty brown scum on potato at room temperature; while Deneke's form, from old cheese, will not grow on potato.

But their morphology and cultural characteristics are not sufficient for the recognition of these organisms. The following specific biological test also must be used. A guinea-pig is first immunized to 1 species of spirillum and a small dose of the organism to be examined is then injected into its peritoneal cavity. If the organism injected is the 1 to which this animal has been immunized those introduced will be rapidly destroyed.

The diagnosis of Asiatic cholera often may (in some epidemics from 25 to 50% of the cases) be made directly from the stools, a smear of which will show vast numbers of these comma-bacilli. Usually, however, it is necessary to inoculate gelatin and agar plates with " rice particles " from the stool. The typical colonies will be recognized in 24 hours if grown on gelatin at 22° C. When but few organisms are present the enriching method of Schottelius is of value. That is, one incubates a large amount of bouillon with a little of the stool and inculcates this for a few hours, in which time these spirillæ will form a surface scum from which cultures may be made. This method is of value in the study of drinking water. To 90 c.c. of the suspected water are added 10 c.c. of a sterile solution of 10% peptone and 5% of sodium chloride. This is incubated in a thermostat and the scum later examined.

In DYSENTERY, RECTAL DIARRHEA, and CANCER OF THE RECTUM the movements are frequent and scanty. They soon lose their fecal character and become mucoid, mucopurulent, hemorrhagic or sero-hemorrhagic. According to the amount of blood present these cases have been classified as " white " and " red " diarrhea. Among the masses of bloody mucus may sometimes be found fragments of necrotic mucous membrane or of cancer. In AMEBIC DYSENTERY, during the acute exacerbations, a diarrhea of from 3 to 6 movements a day is the rule. The stools are thin and watery, offensive in odor, and contain bloody mucus in which the amebæ may be found. These periods of diarrhea are separated by even years during which time the movements are normal or constipated; and yet even in these stools the ameba may be found. It is for this reason that a routine examination of the stools for ameba should be made even in cases without dysentery or symptoms referable to the liver. (This was well illustrated by a case of constipation with irregular fever and without symptoms of hepatic or intestinal trouble. The autopsy revealed a large amebic abscess of the liver.[36])

THE GROUP OF DYSENTERY BACILLI.—In morphology and in some cultural characteristics " Shiga's bacillus " resembles Bacillus typhosus. It is a short organism with rounded ends and is inclined to involution forms. All now agree that it is non-motile. No spores are formed. This organism stains readily in the commonly used aniline dyes, shows a tendency to polar staining and is decolorized by Gram's method.

Since Shiga described his organism 12 other organisms of dysentery have been described, all belonging to 1 group, all with similar morphology and similar staining characteristics, all non-motile, all unable to liquefy gelatin, to form acid from lactose and to form gas from any carbohydrate. They differ in their agglutination reactions to immune sera and in their ability to ferment carbohydrates. Flexner recognizes 3 types: 1. The " Shiga " type, which ferments glucose only. 2. The " Flexner-Harris " type, which ferments glucose, mannite and dextrin, but not lactose. This is the type which prevails in the United States. 3. Bacillus " Y " (His and Russell), which ferments only glucose and mannite.

These organisms cause the so-called " bacillary " or " infectious " dysenteries, diseases which may occur sporadically or in epidemics, *e.g.* the severe epidemics of tropical dysentery. This disease begins as an acute gastro-enteritis with a diarrhea which increases in severity until the stools are very frequent and scanty, lose their fecal character and consist chiefly of mucus and blood rich in these organisms.

In the recognition of the dysentery bacilli the agglutination tests are of greatest value. The blood serum of a patient infected with an organism belonging to the Flexner-Harris type will agglutinate a pure culture of this organism in dilutions of 1 : 1000–1500. In cases of infection with one of the Shiga type the agglutination is less complete.

Pancreatic Disease.—The pancreas may be diseased in part or as a whole. Chronic glycosuria is ascribed to a lesion of the islands of Langerhans. In these cases there is often no evidence of disease of the rest of this organ. In cancer of the pancreas or total obstruction of the duct from calculus leading to atrophy of this organ, the pancreas as a whole is destroyed, but first the tissue which produces its external secretion. There is no quantitative relation evident between the lesion and the impairment of pancreatic function, for the first evidence of pancreatic disturbance may be late, the glandular insufficiency developing all at once as it were. There are no tests for partial pancreatic insufficiency, which have any proven practical, or even theoretical, value.

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[36] See also Councilman and Lafleur, Johns Hopkins Hosp. Reports, vol. 11, p. 395.

The failure of internal secretion has been discussed on page 198. The 3 signs of entire lack of external secretion are steatorrhea, azotorrhea and the lack of the products of putrefaction in a case without diarrhea and without jaundice.

By *azotorrhea* is meant the presence in the stool of a person on mixed diet who has no diarrhea of an unusual number of muscle fibers, some still in bundles, with their striation well preserved.

Steatorrhea.—In some cases of diabetes the stools contain from a few drachms to a cupful of pure yellowish-brown fat. Others consist of about 30% fat. The suspicion of pancreatic disease is certainly justified if a large amount of fluid fat separates itself from the rest of the stool. The simultaneous presence of glycosuria in such cases is rare, the absence of decomposition is not usual nor should it be expected since so much albumin is present, but a simultaneous steatorrhea and azotorrhea are important and with diabetes are conclusive.

And yet azotorrhea may wholly fail in pancreatic disease. Again, steatorrhea alone is not conclusive for with complete atrophy of the pancreas steatorrhea may fail, while it may be present in many diseases other than of the pancreas which affect fat absorption as well as fat splitting. Again, in some cases with an increased amount of fat in the stool the per cent. split is normal.

Atkinson and Hirsh[37] reported a typical case of severe chronic interstitial pancreatitis due to pancreatic lithiasis. The patient, one of diabetes mellitus, evacuated 4 liters of feces daily. The stools were soft or semisolid, leathery-brown in color and had the odor of rancid butter, they contained 54.6% of fat (22.5% neutral fat and 32.1% fatty acid), which was present in lumps varying in size from that of a split pea to that of a walnut.

The assimilation limit for fat for a normal person is about 350 gms. of butter. After meals not exceeding this amount the loss in the stools is not over 7 to 10%. This test should not be made if the person is jaundiced or has acholia due to any other disease; the fat should not be given in an emulsified condition and any diarrhea should be checked with opium prior to making this test.

The attempt has frequently been made to find a preparation of pancreas which will not be affected by the gastric juice. If the administration of such a preparation to a case without diarrhea and with considerable muscle tissue in the stool were followed by a diminution of the muscle fibers, the evidence would be in favor of pancreatic disease. These attempts are partially successful if the gastric secretion be kept alkaline after the ingestion of the pancreon, pancreatin, trypsin, etc.

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[37] Am. Jour. Med. Sci., Oct., 1907.

Permanent Mounts of Small Worms—For the following methods I am indebted to Dr. Thomas R. Boggs.

Boggs' Method.—The worm is allowed to die in water (that it may be fixed while in a relaxed condition). It is then spread out on a piece of filter paper and immersed in an alcohol-glycerin cleaning fluid (alcohol 80%, 16 parts, and glycerin 4 parts). The specimen is allowed to remain in this solution in an open dish loosely covered with cloth or paper, until the alcohol has entirely evaporated off. This may take from 2 to 6 weeks. Since the worm is spread out on the filter paper it will contract but little. Should it do so it may be slightly pressed between slides held together by rubber bands. When the specimen is sufficiently clear it is gently blotted on the slide and covered with melted glycerin jelly. The cover slip is then dropped on and if necessary pressed down until the jelly has hardened. After the jelly is hard the excess is removed from the borders of the cover and the edges sealed with microscope cement or asphalt paint.

The glycerin jelly is made by melting 14 gms. of the best gold mark gelatin in 120 c.c. of hot water and adding 120 c.c. of glycerin. This is then cooled to 50° C., the carefully separated whites of 2 eggs added, the fluid heated gently without stirring and then filtered. The volume is now made up to 240 c.c. by adding water and 1 c.c. of pure carbolic acid added. This jelly is solid at ordinary temperature, but is easily melted under the hot water tap.

To Preserve Stools Containing Parasite Eggs. — The stools are diluted to a soup-like consistency and then 1/10 its volume of formalin added. The specimens are then kept in a tightly corked bottle. Parasite eggs and larvæ will be well preserved.

Flat Worms, Preservation of the Gross Specimen.[38]—To clean the worms the fecal matter is mixed with warm (37° to 40°) normal salt solution sufficient to make a thin broth. If the specimens are obtained at autopsy, the intestinal contents may be washed or scraped off into the salt solution. The worms will move about freely and are easily seen and isolated, especially small worms, such as Hymenolepis nana. With a pair of finely-pointed forceps the parasites are picked up and transferred to a second dish of warm solution. Specimens to be cut in sections may be taken from this solution clean and treated with the proper fixatives (see below). The rest of the material may be placed in 50 to 70% alcohol with or without glycerin, or in Zenker's solution, or in a 2% solution of formalin. Zenker's solution causes considerable shrinking and a rather marked yellowish discoloration. These authors consider the formalin mixture much better as it preserves the natural whiteness of the worms and causes little or no shrinkage.

Preparation of segments for mounting.—The specimens are washed in normal salt solution (0.85%) and fixed by keeping them from 14 to 16 hours in a 2% formalin. They are then transferred to the following glucose medium, which is a slight modification of the Fabre-Domerque medium:

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[38] Mink and Ebeling, U. S. Naval Med. Bull., No. 3, vol. 111.

Sirup (glucose 48 parts; water 52 parts)	1000 c.c.
Methyl alcohol	200 c.c.
Glycerin	100 c.c.
Camphor (q. s. to keep)	100 c.c.

The specimens will clear sufficiently in this medium in 4 or 5 hours. They may, however, be left in it indefinitely. To mount them, a sufficient quantity of glycerin jelly is dropped on a slide and the specimen is transferred to this, care being taken not to admit air-bubbles. A cover-glass which has been passed through the flame finishes the mount. After the glycerin jelly has hardened a few coats of gold-size applied around the cover-glass will furnish rigidity and improve the general appearance of the preparation. Concave slides are desirable when the specimen is of uneven thickness or rather thick throughout.

Preparation and sectioning of material.—To prevent any curling or distortion of the worm or segment a fixative should be used which will kill quickly. For this Zenker's fluid is the best. About 3 or 4 inches of the live tape-worm are taken from the salt solution and stretched out on an ordinary glass slide. By means of a pipet the slide is rapidly covered with Zenker's fluid. The worm will straighten out, harden, and float on the solution. It may then be transferred to a flat dish filled with Zenker's fluid where it should remain for from 2 to 24 hours. By cutting slightly beyond the part needed for work one leaves small end pieces which may be grasped with the forceps in the subsequent manipulations and so the segments chosen for study will not be touched by the forceps. The later processes includes treatment in an alcohol-iodine solution, in graded alcohols for dehydrating, and other steps until the tissue is immersed in melted 45° C. paraffin. In blocking the specimen it seems best to place the longest and broadest surface downward and later trim and mount it as desired. Specimens are best cut either in planes parallel with the long, broad surface, or perpendicular to the long axis. They should be from 25 to 30μ thick. The most convenient stain is a rapidly acting, purely nuclear hematoxylin.

Stained specimens of worms.—The worm, as fresh as possible, is fixed in a boiling, saturated alcoholic solution of mercuric chloride for from 10 to 30 minutes (depending on the thickness of the specimen). It is then washed over night in running water and then in water containing a trace of iodine until it is free from mercury. The specimen is then heavily overstained (for from 12 to 24 hours) with hematoxylin or carmine and decolorized under the lower power of the microscope with acid alcohol until the desired color is obtained. The specimen is then washed, dehydrated in alcohol, cleared in oil of cloves or creosote and mounted in Canada balsam. This method is best for the study of the minute anatomy of tapeworms and flukes. It is not successful with round worms.

CHAPTER V

THE BLOOD

Instruments for Obtaining the Blood.—If a few drops of blood will suffice for examination one usually pricks the lobule of the ear or the tip of a finger, using a simple sharp-pointed lancet, a needle with a cutting edge or an ordinary Hagedorn needle. Special forms of lancet have been invented, some, as the Daland needle, with a guard which prevents the needle from penetrating beyond a certain depth, others with a spring which when released forces the needle to a certain depth (*e.g.* Francke's needle).

If considerable blood is desired a hypodermic syringe should be used to penetrate into the lumen of a vein, preferably the median basilic vein at the elbow. The skin of this region is first made surgically clean, *e.g.*, with tincture of iodine, then a tight bandage is tied around the upper arm and a towel wet with warm bichloride wrapped around the elbow-joint until the needle is inserted. If the blood is intended for quantitative work the bandage should be removed after the needle has been inserted and circulation allowed to return to normal before any blood is withdrawn, since stasis will alter its concentration.

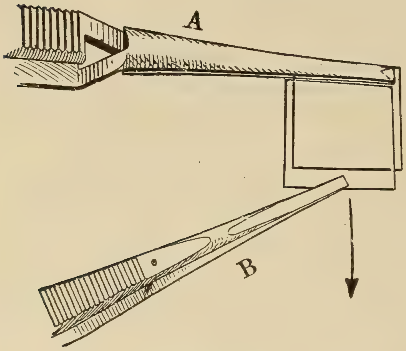

Fig. 105.—Method of making cover-glass preparations.

Two forms of forceps are necessary in making blood smears (Fig. 105). One has crossed blades which will hold the entire length of one edge of a cover-glass firmly and the second, the ordinary straight pinch forceps, has flat smooth points and a weak spring. Cover-glasses should always be handled with forceps since the moisture from the fingers will affect the specimens.

The best glass slides for blood work are of clear glass 1 inch wide and as flat as possible. Those which are slightly curved will rock on the microscope stage unless the specimen is on the convex side.

The cover-glasses should be thin (No. 1, or preferably No. 0) and not over ¾ of an inch square. In general only new cover-glasses should be employed, since it is almost impossible to remove from those once used the little microscopic masses of hemoglobin which latter may be mistaken for malarial pigment, etc.

The glassware must be scrupulously clean. New slides and cover-glasses may be washed in soap and water, then in clean water and lastly in 95% alcohol. Some are so oily that they should be soaked for about 24 hours in concentrated hydrochloric acid, then washed in water, then in 95% alcohol and lastly in ether. The clean glass may be kept either in 95%

alcohol or, carefully wiped (best with an old linen handkerchief), in a dry glass dish. They should be handled only with forceps.

The skin punctured for the blood should not be cyanosed or edematous. We have seen 2 leucocyte counts of blood taken at the same time from the 2 ears of the same person vary by 100% and the same may be said of blood of the 2 hands. The blood is usually obtained from the ear since this is always within reach, the patient cannot watch the worker and the skin of the lobule is relatively painless. If the lobe of the ear is thick it is usually stretched over the index-finger by the thumb and middle finger and pricked on its flat side, but if thin, and especially if several drops of blood are required, it is well to pierce its edge. Many workers prick the palmar surface of the ball of a finger of the left hand where they easily obtain a good drop of blood. Our students studying their own blood search on the anterior surface of the forearm for the pain points and, avoiding these, prick over a small superficial vein. In this way a large drop of blood is easily and painlessly obtained. In the case of very small children the great toe or the heel is chosen. The needle or lancet, washed in soap and water, may be sterilized by dipping it into alcohol. The skin is washed off with alcohol and allowed to dry. The lancet may be driven through the skin by a short, quick, sharp blow; or if several drops are desired, by slow pressure. The patients much prefer 1 hard stab to several ineffectual ones. The skin once pierced should not be squeezed, nor rubbed, nor held in a position which will check its circulation since all these methods to increase the flow will change the concentration of the blood. The drops should well out. The first is wiped off and the second used. In case many drops are to be taken the incision may be wiped occasionally with an alcohol sponge and then with a dry sponge to keep the cut bleeding. Always in advance one should ask for a history of hemophilia and thus avoid hemorrhages difficult to check. In these cases the very slightest prick will furnish even too much blood, although even in these cases blood may be drawn from a vein provided the vein is not wounded (*i.e.*, the first prick successful).

Specific Gravity of Blood—GRAVIMETRIC METHOD.—The gravimetric method of determining the specific gravity of the blood is certainly the most accurate, but requires at least 5 c.c. of blood for an accurate estimation, a good pycnometer and a very accurate chemical balance.

AREMETRICAL METHODS.—A popular method (Roy) since easy, is to introduce single drops of blood into a series of bottles filled with fluids previously prepared with which the blood will not mix and each of a different specific gravity and noting the one in which the drop of blood neither sinks nor rises.

Hammerschlag changes a mixture of benzol and chloroform until it has just the right specific gravity. A glass cylinder, perfectly clean and dry (else the blood will cling to the side of the glass), is filled with the mixture mentioned above, the specific gravity of which is about 1.058. A drop of blood is then introduced, best through a capillary tube bent at the end at right angles so that the drop may be blown into the fluid without imparting to it any upward or downward motion. If the drop rises more benzol is added; if it sinks, more chloroform. After each addition the fluid must be well stirred. It is important to work very rapidly since the specific gravity of the mixture is constantly changing because of evaporation and since there is some exchange between the blood and the fluid which alters the drop of blood. The final result should be confirmed using a fresh drop of blood and working very rapidly. The drop of blood is removed before the specific gravity of the fluid is tested by filtering it through linen. Care must be taken that no bubbles of air are sticking to the drop. Slight differences in the temperature of the mixture make differences in the specific gravity of the mixture which are so great that Langlois varies, not the proportions of benzol and chloroform in the mixture, but its temperature. When the drop no longer rises or sinks he reads the temperature of the mixture and from this reckons its specific gravity.

The specific gravity of the blood serum (obtained by filling a tube with blood, sealing both its ends and allowing it to stand upright until the serum has separated well from the clot) and of the plasma (obtained by centrifugalizing the blood in a centrifuge tube which has been previously washed out with 3% oxalic acid) is estimated in a similar manner.

While the Hammerschlag method looks easy and is simple the possibilities of error are so great that only one with considerable training can use it safely.

The specific gravity of normal blood varies from 1.058 to 1.062 the average for men being 1.059 and for women 1.056 (Ehrlich). The figures given by Piper are, for man 1.055, for woman 1.053 and for children 1.051: Landois, 1.045 to 1.075, the average 1.054: Lloyd Jones, 1.036 to 1.068: and Hammerschlag, from 1.056 to 1.063. Most agree that the specific gravity of the blood of a woman is slightly less than that of a man. That of the blood at birth is 1.066 (Lloyd Jones). It then drops, reaching a minimum of 1.048 to 1.050 in the second year and then rises to a maximum of almost 1.058 (even 1.066). After the menopause the average is 1.054 Diet affects if but little. Menstruation, Schmalz says, is followed by a slight increase. Daily variations are noted by Schmalz, the maximum between 7 and 8 A.M. being 1.060—7, and the minimum from 11 A.M. to 8 P.M. 1.0588.

The specific gravity for the serum and the plasma are about the same, from 1.029 to 1.032, average 1.030. The specific gravity of the plasma, while much more uniform than that of the total blood, nevertheless is diminished in dropsical conditions.

Using the Hammerschlag method, 23 of our students, normal men between 20 and 25 years of age, found their blood to vary from 1.051 to 1.065. In the case of 16 of the 23 it was from 1.057 to 1.061; the mean of all was 1.058.

In pathological conditions the specific gravity of the blood may vary from 1.025 to 1.068, running parallel in most cases to the hemoglobin. It is reduced in all anemias, especially in chlorosis. It is reduced in many cachexias, in which cases the change is in the plasma since the hemoglobin may remain practically normal. It is increased in fevers to from 1.057 to 1.063, in cyanosis and in obstructive jaundice.

Until the introduction of the better forms of hemoglobinometer the hemoglobin was best calculated from the specific gravity. This was especially true of such anemias as chlorosis, in which cases variations in the specific gravity are almost entirely due to variations in the amount of hemoglobin. In cases with hydremia, however, this rule does not hold and yet even in these cases the specific gravity of the plasma is more constant than that of the total blood since the water would seem to be taken up in large part by the red blood-cells. This is true even in severe blood diseases, as, for example, in pernicious anemia.

Dried Residue—HYGROMETRY.—To calculate the dried residue of the blood a weighing glass with ground-glass stopper is first carefully dried and weighed. A little blood is then introduced, the cover put in place and it is again weighed. The cover is now tilted and the blood dried to constant weight (about 24 hours) in a thermostat at a temperature of from 65° to 70° C. It is then again weighed. The solids of the blood of a normal man average about 21.6% of the weight of the fresh blood; of a woman, 19.8%. The figures of Askanazy are: for man, from 20.35 to 22.89%; average, 21.92%; for woman, from 19.58 to 21.46%; average, 20.53%.

It was hoped that this study of dried residue would help in the study of the anemias, since it was found to vary somewhat independently of the specific gravity of the blood, of the red cell count and of the hemoglobin; but its value has not yet been proven.

Sedimentation of the Blood.—The attempt has been made to substitute the estimation of the volume of the red blood-corpuscles for cell counting, since after all it is not so much the number of the red blood-cells as the volume of hemoglobin-containing protoplasm which is important in internal respiration. While this substitution was not successful yet the sedimentation test did win an independent value for itself (see page xxx). The volume of these cells may be determined by the hematocrit (see Fig. 122) using undiluted blood (see page 460), or by the centrifuge using blood diluted with an equal volume of 2.5% potassium bichromate or of Müller's fluid, or, most accurate of all, by the spontaneous sedimentation of the red blood-cells. The difficulty with the method is the varying compressibility of the red cells in different conditions.

Normally the volume of these cells is 50% of the blood volume.

Coagulation.—On few subjects in hematology has so much accurate, careful scientific work been done as on the coagulation of the blood and the results are of unquestionable clinical value. The subject is a difficult one.

There are at least 3 forms of coagulation to consider—that in an open wound, thrombus formation in a closed vessel and coagulation within our laboratory instruments. These processes are very different, and " we cannot bring the appearance of coagulation in the living vessel into direct parallelism with coagulation of blood as ordinarily understood " (Welch), nor can we reproduce the conditions under which either occurs. Thrombosis is a very common complication in typhoid fever, anemia and cachexia, yet the amount of fibrin demonstrable in the blood in these conditions is quite low; on the other hand the blood in pneumonia and in acute articular rheumatism is very rich in visible fibrin and yet thrombosis is of rare occurrence. Again, coagulation in the wound is not a uniform process. More depends on the nature of the vessels cut and on the tissue through which the blood escapes. It is, for illustration, scarcely possible for a man to bleed to death following cross-section of a radial artery, large as that is, while the fatal intestinal hemorrhages in typhoid fever are from vessels so small that they cannot be found without a dissecting microscope. The character of the vessel's wall, the opportunity for it to contract, the character of the tissue of the Peyer's patch and possibly the intestinal contents—all may conspire to make the intestinal hemorrhage much more serious. Then too, the rapidity of coagulation of blood in a tube or glass chamber depends on many factors, few of which are understood. For instance, the longer the blood is in contact with the cut tissues the more rapidly will it clot; blood from a deep wound will clot even 3 minutes more slowly than will that from a superficial wound; each drop of blood flowing from a wound will clot more quickly than will the preceding drops so that if several are allowed to flow the difference in coagulation time between the first and last drop may be almost 10 minutes; the pressure made on the flesh near the cut to encourage the flow of blood, the amount of blood used, the material composing the receptacle in which it clots, this receptacle's cleanliness and temperature, the temperature of the air, and the opportunity there is for evaporation—all these modify the rapidity of coagulation. The above considerations show the need there is of as uniform technic as possible.

Even when the technic is as uniform as possible it is still true that blood removed at the same time from different parts of the body will clot with different rapidity; that the diet and especially the medicines are factors to be considered; and that the coagulation time differs appreciably at different times of the day. The longest time noted is soon after breakfast, when the blood of normal men sometimes clots in from 12 to 17 minutes, a slowness which at any other time of day would be distinctly pathological. We wonder if those surgeons who prefer the forenoon hours for their operations remember this? The most rapid coagulation occurs about 4 o'clock in the afternoon.

While we have not yet been able to gain much, if any, data concerning internal coagulation, as in thrombosis (save the importance of infection), the clotting of effusions, etc., yet the observations made on cases with "the hemorrhagic diatheses," as the purpuras, hemophilias and the anglo-neurotic edemas are of interest. Some surgeons appreciate the importance of the coagulation time in hemophilia and jaundice but many "still take a chance."

A normal coagulation time has chiefly negative value; that is, if it is normal one may feel safe in operating, while if definitely slower than the limits of normal there may or there may not be danger. Hinman and Sladen [1] give the following illustration of this point: The coagulation time in a case of hemophilia was 16⅓ minutes; that is, it was distinctly prolonged and yet the prick in the ear by which this drop was obtained closed at once without further bleeding. On the same day this same ear was pricked a second time. This drop clotted in 18⅓ minutes but this prick bled for 12 hours.

Hemophilia is defined by Howell [2] as a condition limited to the male, the characteristic peculiarity of which is that the coagulation time of the blood is markedly prolonged in consequence of a deficiency in the amount of the contained prothrombin, with the additional characteristic that the defect is transmissable by heredity in accordance with the so-called law of Nasse. Since the prothrombin present in the plasma is furnished by the blood-platelets Howell considers it reasonable to assume that the defect in hemophilia is referable to some functional change in these elements. The antithrombin, which is diminished in cases of thrombosis, is relatively increased in hemophilia owing to the absolute decrease of prothrombin.

In purpura hemorrhagica and other forms of so-called purpura no evidence was found of any variation from normal in either the antithrombin or the prothrombin.[3]

Estimation of Coagulation Time.—"Coagulation time" is not the time it takes blood to clot in a wound, for we have no means of measuring that, but the time which elapses between the appearance in the wound of the drop of blood to be tested and the first evidence of fibrin formation in this blood in the laboratory instrument. One does not measure the time from the moment he gets it in the instrument. For comparable work if drops of blood are to be used all determinations should be made approximately at the same time of day; the blood should always be obtained from the same part of the body; one cannot expect to get uniform flow of blood but he should at least get a free flow; the prick should not be made in the seat of an active or a passive congestion (for tissue lymph and carbon dioxide both hasten coagulation); the second or third drops which well out should be used, not the first and not the later drops; the temperature of the room in which the observation is made should not be unusually warm or cold, although one need not try to control the temperature beyond preventing extremes; and anything which tends to increase the drying of the blood, as a draught of air, should be avoided. In the newer methods from 2 to 8 c.c. drawn from a superficial vein are used.

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[1] Johns Hopkins Hosp. Bull, July 1907, xviii, p. 207.

[2] Arch. of Int. Med., 1914, xiii, p. 92.

[3] For the methods of determining the amounts of antithrombin and prothrombin see Dr. Howell's article.

The method used should allow the observer to prove objectively the presence of fibrin since the drop of blood may dry and appear clotted before any fibrin has formed.

Vierordt's Method.—This method has simplicity to recommend it. A white horse-hair 10 cm. long is boiled in alcohol and ether. A capillary tube 5 cm. long and of 1 mm. bore is thoroughly washed and dried also in alcohol and ether. A drop of blood giving a column about 5 mm. long is received into the tube and the white horse-hair run through it. Each minute the hair is pulled slightly through the drop. The first appearance of coagulation is shown by a slight reddish stain on the hair, which after the blood is well coagulated will again appear clean. It is of greatest importance that that part of the horse-hair which is to come into contact with the blood should not have been touched with the fingers. The amount of blood used should be exactly the same each time, since the coagulation time depends directly upon the amount of blood. All results should be confirmed by a second determination.

Millian's method is a modification of Hayem's. This method which is considerably in vogue among the French is to place a drop of blood on a clean glass slide, cover it by a crystallizing dish to prevent very much evaporation and then at stated intervals to tilt the slide. From the change in shape of the drop of blood, that is, when it ceases to act as a fluid but has elasticity of form, can be determined the coagulation point. Most unreasonable results have been obtained by this method, the coagulation time extending even into hours. The method has been tested under Dr. Boggs' direction in the Johns Hopkins clinic by Hinman and Sladen [4] who found that very much depends on the size of the drop, which should be constant, and on the amount of evaporation. And yet if no better apparatus is at hand this simple method has some value.

McGowan's Method.[5]—Capillary tubes of uniform caliber are filled with blood and small sections broken off at intervals of from 10 to 30 seconds until a fine filament of fibrin is observed between the carefully separated ends of the tube and fragment.

The best method is that of *Russell and Brodie.* Their apparatus consists of a moist chamber with a glass bottom which fits upon the stage of the microscope, while the upper surface is a truncated cone of glass projecting downward into the moist chamber. The lower surface of this is just 4 mm. in diameter on which is placed the drop of blood, care being taken that it only just covers the surface, hence is always of the same size.

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[4] Loc. cit.

[5] Quoted from Ash, Arch of Int. Med., July, 1914, xiv, p. 8.

The glass cone is then quickly fitted into the moist chamber. Through the side of this chamber projects a fine tube, through which, by means of a bulb, a gentle stream of air can be directed against the blood. With the low power of the microscope the cells are watched until they move in clumps.

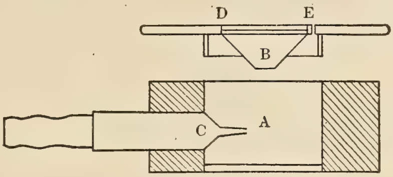

FIG. 106.—Coagulometer of Russell and Brodie as modified by Boggs. A, moist chamber; B, cone of glass the lower surface of which holds the drop of blood; C, side tube; D and E, cover-glass; at E, a pinhole.

This method is the most accurate yet devised. The original apparatus of Russell and Brodie[6] has been modified recently by Boggs who uses a metal tube, an improved glass cone and dispenses with the water jacket (see Fig. 106).

The corpuscles should be agitated as little as possible. They will at first move freely and independently of one another (see Fig. 107, *A*) and then in clumps on the periphery, *B*. As the process of coagulation continues the masses of corpuscles will no longer move within the drop, but the drop changes its shape en masse, the corpuscles showing first an elastic concentric motion, *C*, and finally an elastic radial motion, *D*. That is, the masses of corpuscles will be moved toward the center by the current of air and will quickly spring back to their original position when the current of air ceases. This is taken as the end point since only then can fibrin be demonstrated if the disk be quickly removed and the drop be touched to a piece of filter paper. All clots should be confirmed in this

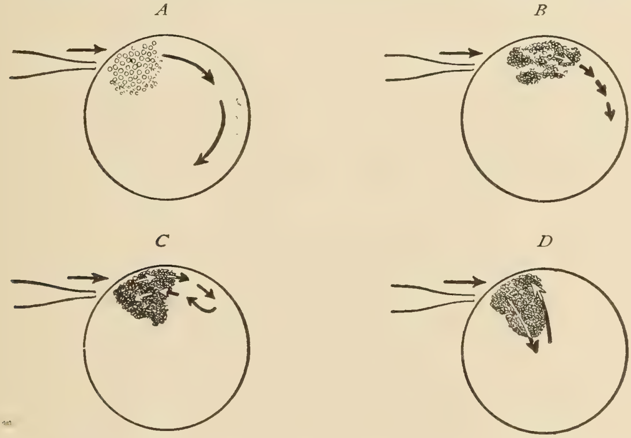

FIG. 107.—Diagram to illustrate the movement of the cells during coagulation.

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[6] Jour. of Phys., May 12, 1897.

way. Sometimes a "vicious circle" is set up in the drop, which clots everywhere but one point where the blood remains fluid. This is the result of too hard blowing. Such a drop should be discarded and another attempt made.

Successive records at intervals of 5 to 10 minutes should not vary over 30 to 45 seconds

In estimating the coagulation time one must take into account not alone the method and the instrument, but also one's definition of the end-point. Using the Boggs instrument, Hinman and Sladen found it to vary from 3 to 8 minutes, averaging 5 minutes and 6 seconds. This is a longer time than some others have reported but they chose a late end-point. (Brodie and Russell reported 3⅛ minutes; Murphy and Gould, 3 minutes, 11 seconds; Pratt, 4 to 5 minutes.) Above 9 minutes certainly would mean delayed coagulation.

Howell's Method.[7]—Two or four cubic centimeters of blood are obtained directly from a vein by means of a sterilized syringe and expelled at once into tubes with a diameter of 21 mm. which had been cleaned carefully with a bichromate acid mixture (see page 15). The coagulation time is the period which elapses between the time the blood is obtained and that when the clot is firm enough to allow the tube to be inverted. Controls are always made using blood known to be normal. The needle should enter the vein at the first puncture since several attempts will allow some tissue juice to mix with the blood which will hasten clotting. A further development of Howell's work is the determination of the prothrombin time.[8] Using these methods Miss Pettibone [9] has made a most careful study of this subject which certainly will in the future have considerable clinical value.

So many methods of such varying value have been used in studying disease conditions that the results are scarcely comparable. Most agree, however, that in hemorrhagic diatheses the coagulation time is immensely increased; to from 10 to 15 or more minutes in certain of the purpuras (due to a deficiency of platelets (Pettibone)) and to even 50 minutes in some cases of hemophilia (due to a deficiency in prothrombin). In long-standing jaundice the coagulation time is increased (due to a calcium deficiency) and any operation should be delayed on such a patient until it has been decreased to about 5 minutes by proper medication. The coagulation time is diminished in venous stasis due to any cause, after repeated hemorrhages (in a recent case following postpuerperal hemorrhages for several days an ordinary blood count could not be made, so rapidly did the blood clot in the capillary of the mixing pipet), after transfusion, by hunger, by too long administration of calcium chloride and by carbon dioxide.

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[7] Arch. of Int. Med., 1914, vol. xiii, p. 80.

[8] Arch. Int. Med., 1914, xiii, 76.

[9] Jour. of Lab. and Clin. Med., Feb., 1918, III, p. 275.

Bleeding Time.—The determination of the bleeding time should be made as a control of the coagulation time since it will detect some cases in which operation might be serious because of hemorrhage and yet the coagulation time normal.

Dukes' method is as follows: The lobe of the ear, cleansed as for obtaining the blood for the coagulation time, is pricked deeply, the time noted, and then each drop as it collects on the skin is picked off on filter paper (care being taken that this does not touch the skin) until the blood ceases the flow. This time seems independent of the depth and width of the drop provided only a capillary area is punctured.

Fibrin Diagnosis.—In very thick smears of blood the fibrin strands may be seen to radiate through the specimen, usually from masses of platelets. These smears after standing for hours under a bell-jar are washed by a gentle stream of water which will remove the hemoglobin, the fibrin stained with fuchsin and the specimen dehydrated and mounted. If examined fresh the specimen should be sealed with vaseline to prevent evaporation. Those diseases in which most fibrin is seen are pneumonia and acute articular rheumatism. In the former case this is suggested as a differential point against tuberculous pneumonia.

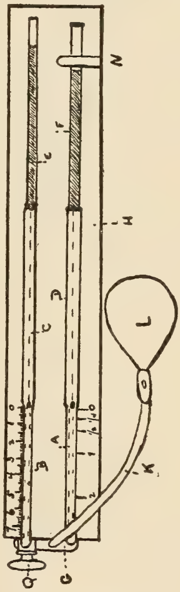

Fig. 108.—Hiss' Viscosimeter.

The Viscosity of the Blood.—The best instrument in use is Hess's viscosimeter. On a base of opaque glass *H* (Fig. 108) are fastened 2 graduated glass tubes, *A* and *B*, which are connected at one end by a third tube, *G*, while this cross tube in turn connects, through a branch, with a rubber balloon, *L*. At their other end are 2 tubes, *C* and *D*, which are drawn to capillaries of very fine caliber, which widen again to the bore of *A* and *B*. The tube *F*, placed on *H*, and held there by the support *N*, is removable and can be replaced by any one of a number of similar tubes. By means of the stopcock *Q* it is possible to establish or to interrupt the communication between *B* and the balloon *L*. The tubes *A* and *B* are bent to a right angle at their junction with *G*. Interposed between the rubber tube *K* and the balloon *L* is a glass tube which by an opening communicates with the air.

The method of making the determination is as follows: In the tube *B-C-D* is a column of distilled water the left meniscus of which is at *O*. The tube *F* is filled by capillary action with blood. It is then placed end to end with *D* and by means of the suction produced by *L* the blood column is drawn up to *O*. The cock *Q* is then opened and by the suction of the bulb the water and blood flow through the tubes *A* and *B*. As soon as the blood reaches 1 suction is discontinued and the readings are made. The body of water which has risen in *B* gives the relation of the viscosity of the blood in question to that of distilled water. The water and blood are now expelled by pressure on the bulb *L*, the cock is closed when the water reaches

O, *F* is removed and the tube *DA* is cleaned by drawing ammonia through it twice.

If the blood is very viscid or coagulates rapidly it may be drawn to ½ or ¼, and the values obtained multiplied by 2 or 4 respectively.

Controls made with fluids of known viscosity are accurate to within 0.5%.

Experiments by Hess showed that with a rise in temperature of 1° C. the viscosity decreased 0.8%. Observations at temperatures of ordinary rooms show an error of about 4%, which is practically negligible. The only corrections of importance are those necessitated by great variations in temperature.

The blood is obtained from the lobule of the ear, which has been previously cleaned with alcohol. If the viscosity of the plasma is to be determined the blood is drawn from the median basilic vein by venepuncture, coagulation being retarded by the addition of dry hirudin, and the blood sedimented.

The viscosity of the blood in health is a variable factor. It is slightly greater in men (4.55) than in women (4.51); it depends on the number of red corpuscles, on the hemoglobin contents, on the gaseous richness, and, to a lesser degree, on the proteid, fat, and salt contents of the blood. And yet it varies directly with none of these factors. The viscosity of the normal plasma varies from 1.7 to 2.0, average 1.86.

The results which Austrian[10] obtained are as follows: The viscosity of the blood and of the plasma are reduced in the anemias, both the primary and the secondary. In leukemia there is hypoviscosity of the blood and hyperviscosity of the plasma. The viscosity of both is increased in polycythemia. Hypoviscosity of the blood and hyperviscosity of the plasma are almost constant in cases of nephritis, the former because of the anemia and the latter to retained products of metabolism. Hypoviscosity occurs often, though not always, in cases with arterial hypertension. In cardiac diseases without edema no constant change is to be found, the coefficient apparently varying with the anemia and with the carbondioxide content of the blood. In cardiac cases with hydremia there is hypoviscosity of the plasma. In diabetes mellitus the viscosity of the blood and that of the plasma are increased. This may be the result of the hyperglycemia, of the lipemia and of the concentration of the blood due to the polyuria. In icterus both that of the blood and of the plasma are generally increased. In typhoid fever it varies with the anemia. It is increased by hydrotherapy and apparently is uninfluenced by diet. The $\frac{\mathrm{Hb}}{(\mathrm{V})}$ quotient is more often decreased than increased. In pneumonia the viscosity is generally above normal.

This may be due to cyanosis and to the retention of salt. Here, too, $\frac{\mathrm{Hb}}{(\mathrm{V})}$ quotient is low. In malarial fever the viscosity of the blood is usually normal or subnormal but is rarely above normal. That of the plasma is normal or increased if hemoglobinemia is present. In no disease can a pathognomonic alteration in the viscosity of the blood be demonstrated.

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[10] Johns Hopkins Hosp. Bull., Jan., 1911, vol. xxii, p. 9.

The Estimation of Hemoglobin.—The estimation of hemoglobin should be the most satisfactory of the blood examinations but the use of faulty instruments has resulted in the accumulation of a vast amount of data of very little value.

It is unfortunate that hemoglobinometers have not from the first been graduated to read in terms of grams per 100 c.c. of blood rather than per cent., for their makers have not agreed what quantity of hemoglobin should be called normal, nor would the same figure be normal for all ages. The blood of a normal child of about 10 years would read but 80% with an instrument standardized to read 100% for a normal man of 30 years, etc.

Another source of error is that many instruments are standardized against dilute water solutions of hemoglobin while hemoglobin in an albuminous fluids like the blood-plasma will give higher readings; hence in reading the blood in extreme anemias we get misleading figures.

There are at present but 2 instruments to be recommended, Miescher's hemoglobinometer and Sahli's hemometer, but so much of the past work has been done with other instruments that we will describe briefly a few of the more popular ones in order that the student may read the literature of medicine with better understanding.

Miescher's Hemoglobinometer. — For years the Fleishl instrument was the best and this later was improved in some details by Miescher. Miescher's instrument is suitable for the laboratory and clinic only since it is expensive, bulky, requires a dark room, considerable time for each determination and considerable practice. The blood is diluted in a beautifully made mélangeur (see Fig. 109), which allow dilutions of 1 : 200, 1 : 300 or 1 : 400. These pipettes are particularly well marked, each small line on either side of the main lines indicatng 1/100 of the length of the entire column thus saving the time necessary to bring the blood column exactly to one mark.

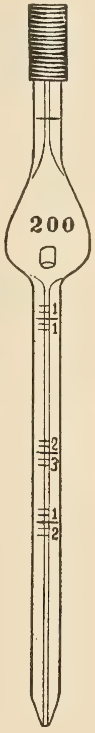

Fig. 109. — Mixing pipette of Miescher's hemoglobinometer.

A large drop of blood is aspirated to the point indicated for the desired dilution and then the pipette is filled with a 0.1% sodium carbonate solution (A stock solution of 10% is diluted 100 times.) The dilution chosen should be such that the readings will be made near the middles of the color-prism. The pipette is handled exactly as for a blood-count (see page 456). One side of a cell (of which there are 2, one 15 mm. and the other 12 mm.

in depth) is filled with water. None should leak into the other half. The blood, well shaken is then blown into the other chamber of this cell. Both the water side and the blood side should have convex meniscuses. The cover-glass *E* is then slid in place pushing off the excess of fluid and leaving the chambers exactly full. The small cap, *F*, will now hold the cover-glass secure and also limit the field of vision. The cell is inserted in the receptacle on the stand, *A*, and the instrument placed in a screen which admits light, a yellow flame, whether from gas, oil, or candle, at one point only, where it will fall directly on the mirror and illumine both fields equally. Electric light, a gas-light with a mantle, or sunlight, cannot be used. The observer sits in a comfortable manner with the eyes about 25 cm. above the instrument and makes his observations with both eyes open rotating the milled head *C*, which moves the color-prism, until that part of the prism (see Fig. 110) which just matches the color of the blood-mixture is under the water-half of the cell. Since the retina is soon fatigued the eyes should be rested each 15 seconds. At least 5 different readings should be taken and the mean, not the average, used. The blood is then transferred with the mèlangeur to the shallow chamber and a similar series of readings is made for control. Since these cells have heights which are to each other as 5 is to 4 different parts of the color-prism will be used. The average of the readings with the lower cell multiplied by 5/4 should not differ from the average made with the higher by over 2%.

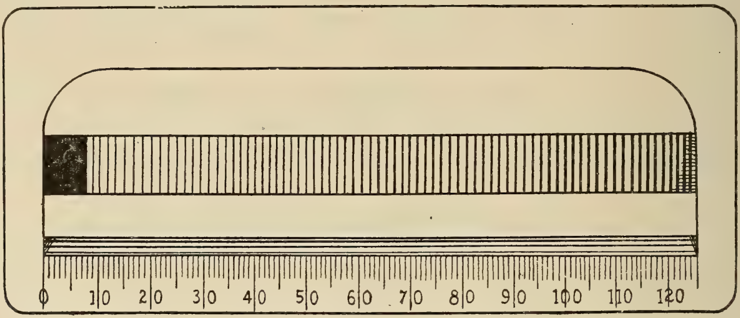

FIG. 110.—Color-prism of the Fleischl-Miescher instruments.

Each instrument is accompanied by a scale which gives the number of milligrammes of hemoglobin per liter of diluted blood corresponding to the readings of that particular instrument. It is then easy, making due allowance for the dilution, to determine the number of grammes of hemoglobin in 100 c.c. of blood. If then, with due observance of the age curve, the worker wishes to express his answer as a percentage, he is at liberty so to do. This instrument is claimed to be correct within 0.2% of hemoglobin.

The mélangeur is cleaned, etc., just as is that of the blood-counter.

Fleischl's Hemoglobinometer.—The pipette of the older Fleischl instrument was a small short cylindrical capillary tube (see Fig. 111), which held from about 5 to 8 cmm.

of blood. This was filled by just touching 1 end to a large drop of blood until the surface of the fluid at the ends is flat. Meanwhile, 1 side of the cell (see Fig. 112, *H*) of the instrument has been filled with water and a few drops of water placed in the other side. The pipette filled with blood is dropped into this latter side of the cell and emptied by rapidly agitating it in this water; and then washing any blood which may cling to the pipette back with a few drops of water from a medicine dropper. More water is then added and the whole well mixed by means of the handle of the pipette until this chamber of the cell is filled to the brim. The upper surface of fluid in the 2 halves of the cell should be just flat. They may be covered over with a suitable cover-glass. The instrument is read in a dark room, as is the Miescher.

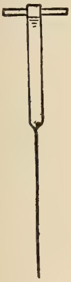

Fig. 111.—Pipette of the Fleischl instrument.

There are a few precautions to observe. The images of the 2 halves of the cells should fall on the right and left halves of the retina, never on the upper and lower, since the lower half of the retina is not nearly as sensitive as is the upper; the light should be at the side, never in front of the instrument; as small a candle as possible should be used; if there is no screen handy, a tube of dark paper will suffice to cut out extraneous rays. The inconveniences of these machines are that they use a color prism which necessitates accurate standardization and a light of a constant color value.

Gowers' Instrument.—This little instrument (see Fig. 113) was for years the best the general practitioner had. It was cheap, easily portable, simple and when well made, fairly accurate. It consists of a color-tube. *B*, containing a fluid with the tint of a 1% hemoglobin solution; a graduated test-tube, *A*, and a pipette *C*, which will measure 20 cmm. of blood. The blood obtained in the pipette is diluted with water in the tube, *A*, until its tint matches that of *B*. The percentage is read directly on the graduated scale from the height of the diluted blood.

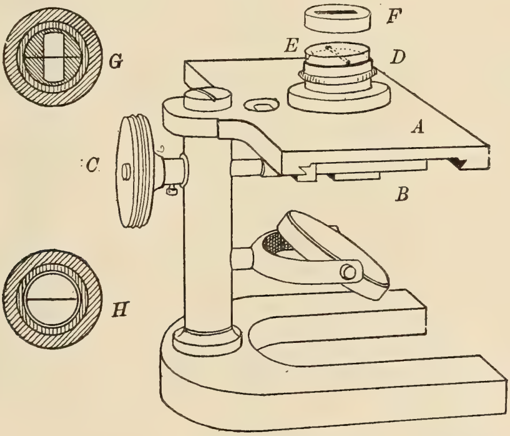

Fig. 112.— Miescher's modification of Fleischl's hemoglobinometer. *A*, stage; *B*, color-prism rack; C, Milled head; D, Cell; E, Cover-glass; F, Cap; G, Cell seen from above; H, Cell of Fleischl's Instrument.

The color tubes contained gelatin stained with picrocarmine and so illumination of constant color value must be used. Each instrument had a tube to use in sunlight and another for gaslight. When not in use these tubes should be protected from sunlight.

The Hemometer of Sahli (see Fig. 114).—For every-day clinical work Sahli's hemometer is the best on the market. It is similar to Gowers' hemoglobinometer except that the color-tube contains a 1% solution of acid hematin, a pigment which is quite constant in composition and color value and the hemoglobin of the blood to be tested also is changed to acid hematin by hydrochloric acid. This instrument may be used in any light since the 2 tubes contain the same substance and would therefore be modified by different lights equally. The blood, obtained in a graduated pipette holding 20 cm. (see Fig. 113, *C*), is blown into the graduated test-tube which previously had been filled up to the 10% point with a $0.1N$ HCl. (This may be made with sufficient accuracy by diluting 15 c.c. of the pure acid to 1 liter with distilled water. Sahli recommends that a little chloroform be kept in this stock bottle.) The tube is thoroughly cleaned of the blood by sucking up and blowing out the acid several times. The hydrochloric acid will in a few minutes change the hemoglobin to acid hematin. It is then diluted with distilled water (mixing it well after each addition by covering the tube with the thumb and inverting it several times, then wiping back any fluid clinging to the skin by drawing the thumb across the mouth of the tube) until its tint corresponds to that of the standard color-tube. The instrument is provided with a very convenient little stand with a ground glass back which renders the reading easy and quite accurate.

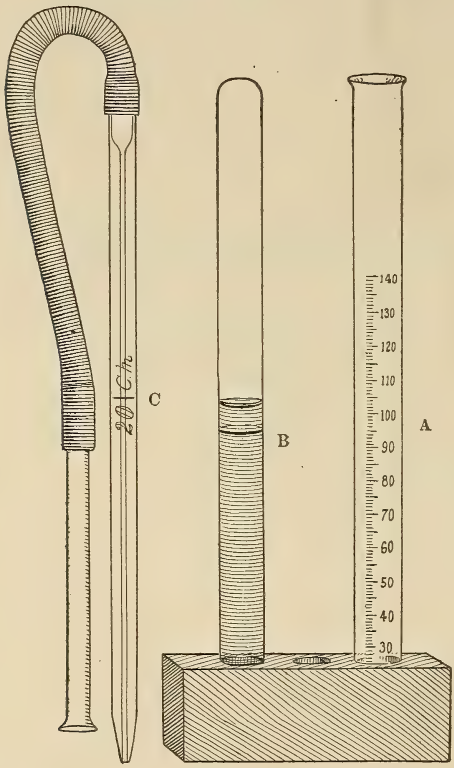

FIG. 113.—Gowers's hemoglobinometer. A, graduated tube; B, color-tube; C, pipette.

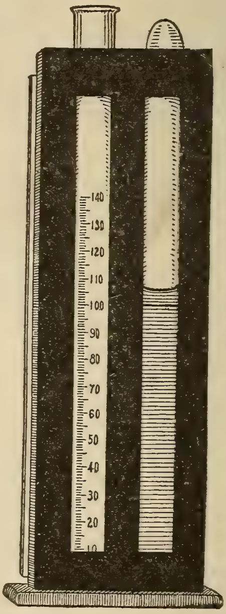

FIG. 114.—Sahli's hemometer.

The color-tube certainly does deteriorate with age and so should be restandardized frequently.

Dare's Hemoglobinometer.—This instrument (see Fig. 115), which has won a deserved popularity, compares a film of undiluted blood with a color-prism stained with golden purple. The pipette (see Fig. 116) consists of 2 plates of glass, 1 white, *A*, 1 clear, *B*, between which is a slit of known width. A rather large drop of blood is necessary and will at once by capillarity fill the slit. The pipette is then at once slipped into the instrument (Fig. 111, *B*), and the reading made before the blood can clot, using the light of a candle, *E*. The telescope tube, *A*, allows accurate focussing and also an advantageous magnification of the 2 color-fields. The prism is rotated by means of a small wheel, *D*, until the colors match and then the reading is made at the edge of the disk. The advantages of this instrument are that undiluted blood is used; that a determination takes but a few seconds; that leucocytes do not affect the reading as in the other instruments; and that it can be used in a light room. We have found that the readings of the same blood made by several persons with different instruments have compared very closely.

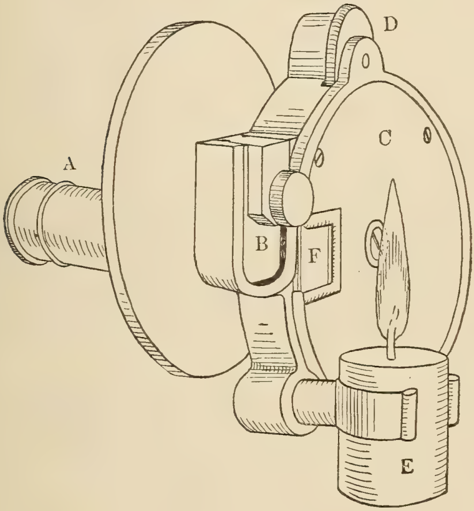

Fig. 115.—Dare's hemoglobinometer. A, telescope; B, pipette in place; C, case inclosing color-prism; D, milled head moving prism; E, candle; F, window admitting light to color-prism.

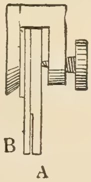

Fig. 116.—Pipette of Dare's instrument. A, the white glass; B, clear glass disk.

The Tallqvist Scale.—This simple little book of blotting-paper with a scale of colors, exploited as a great boon to the general practitioner, has done great harm to the interests of accurate clinical work. The instrument can be carried easily in the pocket and a determination made in less than a minute. The colors of the scale vary by 10%, therefore any intermediate percentage must be estimated by the eye. We admit that an eye trained to use more accurate instruments will soon use this color-scale with some degree of accuracy but such a person would prefer to guess without its doubtful aid.

The spot of blood is obtained by holding the edge of the blotting paper against a large drop of blood and then at once blotting the paper by squeezing it between 2 pages of the book until the luster of the blood spot is lost. The reading is then made by reflected light before the drop becomes dry.

In hospital work we find it a great advantage to have several varieties of hemoglobinometers in use and to insist that each student shall use them all. Only in this way can he learn to appreciate the strong and weak points of each. The man who uses but one soon places undue reliance upon its accuracy. Let him use 2 of different makes and find that they differ from 5 to 20% and he will appreciate the difficulties involved.

We have used a Miescher as standard and have required each clinical clerk first to standardize the instrument assigned him, which may be 1 of 7 different types, against this and in future work to make the necessary correction. Later we have required the student to correct their instruments against several accurate blood counts on normal persons so that the reading of 5,000,000 red cells should be 100% (*e.g.*, if the count is 5,200,000 and the reading 85, then $5{,}200{,}000 : 5{,}000{,}000 :: 85 : x = 100$. Of course several such tests should be made.)

These instruments very seldom read 100% for the blood of a normal person. We have careful records of 176 medical students, all normal men during the third decade of life. The Fleischl instrument used in 161 cases read from 65 to 110%; of these, 136 varied from 80 to 100% and 52 from 90 to 95%; the mean was 92.5%. Of the 156 records made with the Dare instrument, which varied from 65 to 110%, 105 varied from 90 to 100% and the mean was 95%. Of the 150 records made with the Gowers', which varied from 70 to 120%, 81 stood between 90 and 100% and the mean was about 92%.

The blood of 125 students was examined using the Miescher instrument. (Practically all of these estimations were controlled at the same hour of the following day.) The results varied from 11.4 to 17.6 gms. per 100 c.c. It was almost impossible to determine the mean of these figures, so uniform was their distribution. Their average was about 14.5 gms. Considering this 100%, the blood of 38 students varied from 90 to 100% and 21 from 100 to 105%. (Note how different the readings on the other instruments.) The range of variations with the Miescher seemed wide, yet they ran more parallel to the blood-counts than did the results with other instruments.

Hemoglobin.—One hundred cubic centimeters of normal blood, it is usually stated, contain from 13 to 14 gms. of hemoglobin. Careful estimations of the hemoglobin at the various ages have shown that there is a definite curve which runs quite parallel to that of the red blood-cells.

Age	Gms. per 100 cc. of Blood
1 to 4 days	19.329 to 21.160
8 to 14 days	17.869 to 16.124
8 to 20 weeks	15.362 to 12.928
6 months to 5 years	10.971 to 11.373
5 to 15 years	11.151 to 11.796
15 to 25 years	13.034 to 13.870
25 to 45 years	14.727 to 15.013
45 to 60 years	12.484 to 13.150

From this table of Leichtenstern (modified from Sahli) it is at once evident that the age curve must be considered in all blood-work, and that it would be better were the hemoglobin estimations given in grams per 100 c.c. rather than in percentage, since there is no one figure which could be considered 100% for all ages

By *oligochromemia* is meant a relative diminution in the amount of hemoglobin per unit volume of blood. It therefore is a relative and not an absolute value.

THE EXAMINATION OF THE FRESH BLOOD

The examination of the fresh blood in every possible case is a matter of routine which will take but about 3 minutes and may save a great deal of time. In the majority of cases not seen in the office we must rely on stained specimens, although the fresh blood would give more information, sometimes unexpected and of the highest value (see page 658), but more often hints which suggest to the worker along what further lines of examination to proceed. It should also be a matter of routine to make a few dried specimens whenever one examines a fresh specimen or counts the blood. These need not be stained but can be filed away. Later, because of the further developments of the case, those old smears may prove of greatest interest.

Technic.—To make fresh blood specimens, the slide and cover-glass must be perfectly clean (see page 463). The slide should be warmed by rubbing it rapidly with a cloth or by holding it for an instant near a flame, since the blood spreads much better on glass warmed to about body temperature than on cold glass. The skin is punctured, the first drop wiped off, and the second or a later one, when about 2 mm. in diameter, is picked up on the cover-glass held in the pinch forceps, care being taken that the cover-glass does not touch the skin, and this is then dropped onto the slide. The drop should be so small that when well spread the blood film hardly reaches the edge of the cover since the distribution of cells varies at different parts of the spread and so one should be able to examine the entire specimen. The blood should spread evenly. Under no condition should pressure be made on the cover-glass to aid the blood to spread since this will make a poor specimen only worse. Neither should the coverglass be pushed into a better position. The student should always be careful to drop the cover-glass on the convex side of the slide, and thus avoid a rocking specimen.

Red Blood-cells.—In the well-made specimen the red corpuscles will all lie singly, flat on their sides, not overlapping, nor in rouleaux. If, as sometimes is the case, it is important to know whether the tendency to rouleaux formation is increased or diminished a larger drop of blood is used.

The *number* of the red blood-cells may be estimated with a certain degree of accuracy by one who always uses approximately the same sized drop of blood. The *shape* of the redblood-cells is of considerableimportance. In the circulation they may, as is claimed, be cup-shaped, but in a well made specimen they flatten out on the glass as perfectly round or, where more crowded, polygonal biconcave discs. In badly made specimens, and sooner and later in a good one especially along the edge, many of the cells are crenated, that is, are spherical and covered with small prickly points. If a cell has but one of these points and that is on the flat surface of the corpuscle it may be mistaken for a small ring form of the malarial parasite. By crenation, however, is meant not alone this artefact of prickle formation but more especially a pathologic change in the contour of the corpuscle while in the circulation. Instead of being a round disc with a circular edge these cells have an uneven, shrunken margin. This is seen in the cells harboring the parasites of quartan and æstivo-autumnal malaria.

The presence of *poikilocytes* is important in diagnosis. Poikilocytes are corpuscles which seem to lack that remarkable elasticity of shape which keeps them always round except when under direct pressure and so they are flabby and assume many odd shapes. Some are indented, some are ellipses, crescents or ovals while others have the well known sausage and battledore forms.

Poikilocytes may be due, first, to technic. Pressure on the cover-glass will cause a certain number of the corpuscles to break up into small spherical masses and small elongated rods which resemble bacilli. The ease with which this occurs will depend to a great extent on the condition of the corpuscles. If, because of disease, they are " weak," a slight injury will affect them more than normal corpuscles (Stengel). Any motion of the cover-glass after the cells have spread will distort them considerably. Second, heat will produce poikilocytes. If a specimen of fresh blood in a moist chamber be heated to from 50° to 54° C. the cells will present a most remarkable picture. The corpuscles lose their shape and show definite contractile movements. Some will elongate considerably and move around with a vermicular motion. We have known a whole hospital staff to study with astonishment the gyrations of these overheated red blood-cells, confident that some new parasites had been discovered. More commonly corpuscles when heated will bud, these buds become detached and swim in the serum as microcytes. Sooner or later in such a specimen nearly all of the poikilocytes will break up into fragments. Third, poikilocytes will increase the older a blood specimen gets. One assumse that red corpuscles are living cells, (although some believe that they die when they lose their nuclei before functioning as blood corpuscles) and that poikilocyte formation under the microscope is evidence of death changes which appear much earlier than normal in the blood of some patients. These changes are best studied in well-sealed specimens on a warm stage, and resemble those of the over-heated specimen except that they are less in degree. But, finally, the poikilocytes which interest us most are cells which are misshapen in the circulation and so are seen in the quite fresh blood. A very few may be found in fresh normal blood, but many are present in that of any very severe anemia and especially in primary pernicious anemia of even mild

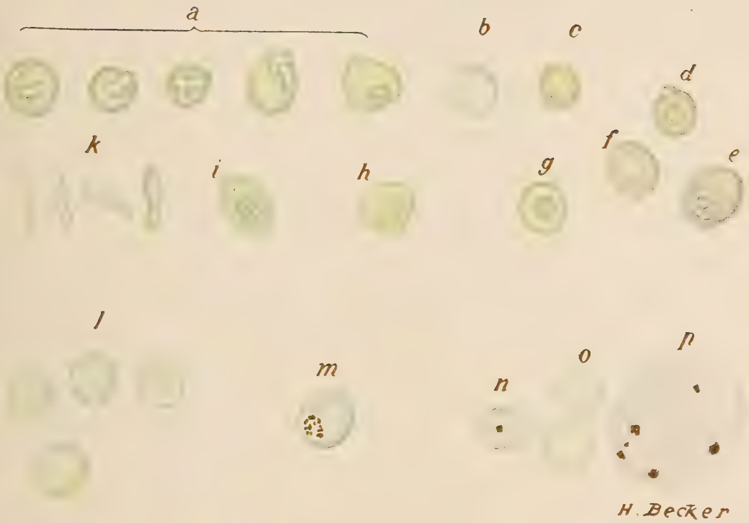

FIG 117..—Fresh blood. *a*, cells with Maragliano's endoglobular degenerations; *b*, cell containing a navicular body, from a case of measles; *c*, the bacillus-like degeneration; *d*, a Maragliano degeneration in process of extrusion; *e*, a form of "hemoglobin degeneration" giving a dark area; *f*, like *a*; *g*, like *e*; *h*, a degeneration like *e* but almost free from cell; *i*, a pseudo "segmenting parasite"; *k*, an "ameboid" microcyte; *l*, estivo-autumnal hyaline malarial parasites; *m*, a full-grown estivo-autumnal parasite, and, *n*, a segmenter, both found in the peripheral blood; *o*, same as *l*; *p*, macrophage from a case of pernicious malaria filled with malaria parasites. × 900.

grade. Of the many forms 2 were once supposed to be characteristic of this disease, those resembling a battledore and the elongated or sausage forms. Poikilocytes would seem to have ameboid motion; at any rate they changed their shape. This is best seen in the small ones. (Plate 1, 23–28; Fig. 117, *k*). The poikilocytes of anemia are probably immature forms rather than injured cells.

The *elasticity* of red cells certainly varies; in lead poisoning it is said to be increased. The large pale cells in anemia on the other hand look flabby.

The projection of the *budding red corpuscles* may have the color of the normal cells or be paler or darker. They are attached to the cell by a longer or shorter pedicle and often break loose. A former and mistaken theory of the origin of blood platelets was that they are free buds of red cells free of hemoglobin.

The *size* of the red blood cells should be noted. Normally in the adult, these cells have a quite uniform diameter which averages 7.5μ. One always finds a few microcytes and some cells fragmented or injured by his technic. In the normal infant's blood there is much greater variation in size. In chlorosis these cells show quite a uniform diminution in size while in pernicious anemia cells of all sizes may be present yet the majority of them will be larger than normal. In secondary anemia they vary much in size and yet many will be normal and many others smaller than normal. In tertian malaria the infected cells are large, swollen and pale, while in quartan and æstivo-autumnal malaria the infected cells are small and shrunken. The average size of the red cells is said to be increased in jaundice, cholera, lead poisoning and leukemia; also in congenital heart disease and in cretinism.

The *color* of the corpuscles is normally greenish-yellow. In the great majority of cases the variations in color are quantitative rather than qualitative and due to differences in the amount of hemoglobin; that is, to the thickness of the corpuscles. The thickness of a corpuscle is easily estimated by the appearance of the cell at its center. While the biconcavity of a a normal cell can be made out only by accurate focussing that of a " light weight " corpuscle is very apparent while some cells are so thin that the hemogoblin cannot be seen at their center and they appear as narrow rings, the so-called "pessary forms." On the other hand, some corpuscles seem to lack a biconcavity while others, especially thc microcytes, appear even biconvex.

Some red corpuscles show a quantitative change of color, due apparently to some chemical change of the hemoglobin. For illustration, the corpuscles which contain quartan or estivo-autumnal parasites appear much darker than the other corpuscles and also have a greenish or " brassy " tone. A similar, although less marked, change in color is seen in some microcytes, in cells fragmented by mechanical injury and in red cells engulfed in phagocytes.

In some diseases the cells show a quite uniform change in color. In chlorosis, for instance, nearly all of the cells are paler and in pernicious anemia many will seem darker than normal. In other conditions it is the variation in color of the cells which is important, as in secondary anemia and in malaria. It is for this reason that the examination of the fresh blood is more valuable in diagnosis than that of stained specimens for in the latter much of color value is lost.

Nucleated reds are often quite conspicuous in the fresh specimen. An occasional normoblast is seen in normal blood but it is a pure anomaly.

The *partial degenerations* of the red blood-cells are very important evidence of the intravascular health of the cells as well as dangerous sources of error in the diagnosis of malaria (Fig. 117). These are necrobiotic changes which appear sooner or later according to the intravascular condition of the blood and the treatment it receives when or after the specimen is made. The areas in the cells showing these changes have received a variety of names, as vacuolization, pseudo-vacuolization, pseudo-nucleation, état cribriform, globular decolorization, but the name most commonly used is "Maragliano's endoglobular degenerations." These appear in normal blood as a rule in from 30 to 70 minutes after the specimen is made. Usually they develop near the center, but sometimes near the periphery, of the cell. One cell may contain one or several. The corpuscle appears thinner at one point and a vacuole-like area appears which seems free from hemoglobin. This area is usually round, although it may be elliptical, and increases in size until a mere rim of hemoglobin-containing protoplasm may be left. Although these spots resemble vacuoles they probably are areas of coagulative necrosis which may be extruded from the cell or remain visible when the rest of the cell goes to pieces. These areas certainly change their shape and their position within the cell, their rapid motions resembling those of malarial parasites. This is not true ameboid motion, however, but due probably to changes in the dying protoplasm around them. The rapidity with which these degenerations appear in the specimen, other things being equal, will depend on the intravascular condition of the corpuscles. Maragliano and Castellino doubtless exaggerated their importance in diagnosis and prognosis, yet they are most conspicuous in severe cases of disease and are especially numerous in the primary anemias. These vacuole-like areas in the fresh blood and even more so in stained specimens may be mistaken for cell nuclei or for malarial parasites and explain many a mistaken diagnosis. Only the trained eye can distinguish them from the hyaline forms of the malarial organism, from which they differ in that they grow larger and more numerous the longer one searches for them. They occupy as a rule the center of the cell, are round or oval in shape, and, what is most important, they are too lens-like and enlarge or diminish in size as one changes the focus while a parasite would become less and less distinct; in general they are much easier to see than is a parasite; their movements may simulate those of an ameboid organism and their periphery may show the same wavy motion, but this is not true ameboid motion since they do not change their position by means of their change of shape. Some resemble beautiful " segmenters " (see Fig. 117, *i*). In Fig. 117 the attempt was made to show these differences (contrast *a*, *f*, and *o* with *l*). In fixed specimens they show a granular structure and will take a basic stain.

Their lack of constancy in size, the changes in their appearance on changing the focus and the absence of a distinct membrane and chromatin net-work of the nucleus should differentiate them from nucleated erythrocytes.

Some special forms of endoglobular degeneration deserve particular notice; some which have a definite crescent shape are famous since twice described as the parasite of measles and more recently as that of spotted fever (see Fig. 117, *b*).

Some rod-like areas resemble bacilli (*c*). These may keep up a constant vibratory motion, moving practically through the whole substance of the cell and looking and behaving remarkably like a motile organization.

Some present the appearance of a small dark cell on top of a larger and paler one, although focussing shows them to be in the same plane (see Fig. 117, *e*, *h*, *g*, Ehrlich's hemoglobinemic degeneration). Another example of this degeneration is well illustrated in æstivo-autumnal malaria, cells whose hemoglobin is gathered in a mass around the parasite (see Plate V, *o*), the rest of the cell colorless. This degeneration is best seen in pernicious anemia.

Granules which would seem to be remnants of the nucleus and described by Vaughan [11] may be studied in fresh blood stained with Unna's polychrome methylene blue. To make the specimens the ball of the finger is well cleaned with alcohol and ether. On it is then placed a drop of the stain and the skin pricked through this drop in order that the cells may come in contact with the stain before they do with the air. A drop of the mixture of blood and stain is transferred to a slide and covered at once with a cover-glass. In a few minutes a few cells may be found containing violet granules which are coarse or fine, some in a line reaching across the cells and others connected by a filament. There is a remarkable constancy in their occurrence. In normal adult blood they are found in from 0.5 to 1.8% of the red cells and in almost exactly the same percentage in a variety of diseases which have little influence on the blood. In the blood of the new-born, they are found in from 1 to 7% of the cells; in that of a fetus 2¼ inches long, in 24%. In the anemias they are more numerous, especially in primary pernicious anemia in which cases even 18.8% of the cells may contain such granules. Their number in general runs parallel to that of nucleated red cells. Vaughan gives as reasons for thinking that these granules are remains of the nucleus that they are not artefacts, they occur especially in normal-looking cells, are situated in the position of the nucleus and are increased in conditions in which nucleated reds appear. He suggests that they may be a more delicate sign of anemia than are nucleated reds.

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[11] Jour. of Med. Research, 1903.

Morris [12] has called attention to granules which are nearly always single and round, which are sharply circumscribed, eccentrically placed and which have the same staining reaction as the nucleus. These are almost certainly nuclear fragments. They are found in the blood of the human embryo, in the anemia of infancy and in those conditions of the adult in which other and clear evidences of blood degeneration are present, as pernicious anemia, secondary anemias, chronic myeloid leukemia, etc.

Various poisons, potassium chlorate, pyrogallic acid, *et al.*, often produce in the red cells vacuole-like areas or clumps, which are motile and which may break free from the cell, or which may be left free when the cell disintegrates. Heinz and Bloch describe these as "areas of poisoned protoplasm."

Leucocytes.—The presence of a leucocytosis and its character will often be suggested by the fresh blood examination, although such suspicions should always be confirmed by an actual count. To get a good idea of the leucocyte picture the fresh specimens should be carefully made since the distribution of the white cells varies somewhat in different parts of the specimens and so the entire specimen must be studied.

The leucocytes in fresh specimens appear as colorless, nucleated, ameboid or immobile cells, which do not float in the current with the red corpuscles.

In the FRESH SPECIMENS the following leucocytes may be recognized. *Small mononuclears* which are cells about the size of a red corpuscle, some a little larger others smaller, with a nucleus relatively large, round as a rule although sometimes deeply notched and central in position. Their protoplasm is scanty. In some it is hardly seen while in others it presents a ragged edge around the nucleus and may appear somewhat granular. In certain conditions, *e.g.*, in lymphatic leukemia, cells of this type are said to be ameboid and this is suggested by a study of these cells in tuberculosis. Normally, these average 32.2% of the leucocytes.

Large Mononuclears and Transitionals. Endothelial Leucocytes.—These, as seen in fresh normal blood, vary in size from that of a lymphocyte from which they can scarcely be differentiated in fresh specimens to others several times the size of a red blood-cell. The nuclei of these larger, very characteristic cells is sometimes round but more often oval in shape, eccentric in position and sometimes deeply notched (the "saddle-bag" or the "wallet-shaped" nucleus). The protoplasm is very abundant and is clear. Although these cells appear non-ameboid yet it is interesting that in malaria they clearly are phagocytes. They average 7.2% of the total number.

Ehrlich applied the term transitionals to those cells of the large mononuclear group which have deeply indented nucleus (see page 469). They would seem, however, to be merely the older forms of the endothelial leucocytes with which they are now counted. They are the largest of all blood cells. In their abundant protoplasm may be seen a few granules near the nucleus.

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[12] Arch. of Int. Med., March, 1909.

Polymorphonuclear Finely Granular Cells.—The finely granular cells of Max Schultze average 58.5% of the total number of leucocytes. In the circulation they are from 10 to 15μ in diameter, but on the slide their apparent size is much larger and depends chiefly upon the extent to which these spherical cells are flattened out upon the glass. Their protoplasm is filled with fine dust-like granules. The nucleus has the shape either of a bent rod, a skein of fibers or of several masses of chromatin (hence the old name "polynuclear cells"). These when they leave the blood-vessels are the ordinary pus-cells, the greatest phagocytes of the body.

The polymorphonuclear coarsely granular cells of Max Schultze, the *eosinophiles* are usually a trifle smaller than the preceding. Their nucleus is possibly less polymorphous but their protoplasm is filled with coarse, blackish, very refractive round or slightly oval granules of quite uniform size and shape, and about 1μ in diameter. These are the most ameboid cells of the blood, and average 1.6% of the leucocyte count.

The *Mastzellen* in the fresh specimen resemble the coarsely granular cells. While they cannot with certainty be recognized yet their granules vary much more in size and their nucleus is often trilobed. These cells average in normal blood 0.5μ of the total number.

Pigmented leucocytes are best studied in the fresh or air-dried specimens. The pigment is sometimes from malarial parasites (melanin) and is very important in the diagnosis of malaria, or it is pigment picked up by the leucocytes in other conditions as in melanosarcoma.

Hemosiderin pigment as ochre granules is seen, although rarely, in the leucocytes of cases in which there is rapid blood destruction. The iron of this pigment may be demonstrated by treating the smear first with 2% potassium ferrocyanide and then with 0.5% hydrochloric acid. The specimen is mounted in glycerin. These granules will take on a blue color.

Blood-dust or *hemokonien granules* is the name given by Müller to the very fine granules which dance actively between the red corpuscles in fresh normal as well as pathological blood. Finding them in large numbers in a case of Addison's disease he supposed that they bore some relation to that malady, but later found them present in all bloods, although in very varying amounts. They are small, round, colorless granules, which vary considerably in size, some even 1μ in diameter resembling micrococci but the most much finer and dust-like. They are best seen by gas illumination. He found that they did not give the osmic acid test for fat, nor were they cleared by acetic acid, as albuminous granules would be. These granules were further studied by Stokes and Wegefarth[13] who decided that they were the free granules of leucocytes. Their reasons for this opinion were: that in man they resemble the leucocyte granules in size, being both coarse and fine, while the horse and rabbit which have peculiar granules in the leucocytes have similar blood-dust granules; that they can be seen to escape from the leucocytes if certain reagents are added to the blood; and, lastly, the larger ones take an eosin stain. These granules are supposed to bear some relation to immunity. Doubtless all the so-called " spores " described in the blood are hemokonien granules.

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[13] Johns Hopkins Hosp. Bull., December, 1897.

The *fat globules* of the body plasma appears in fresh blood specimens as exceedingly fine dust-like granules which would easily escape observation. They form a perfect cloud in the plasma in cases of lipemia.

The *blood platelets* in fresh specimens appear either singly, in clumps, or as masses of amorphous granules in the periphery of which are vacuole-like areas containing a watery fluid, the so-called " granular masses of Max Schultze." At this point we would remind the reader that all platelets in fresh blood specimens will at once stick to the glass and soon disintegrate and that a floating object in the plasma certainly is not a platelet however much it may resemble one (see page 525).

The large *macrophages* can be studied only in fresh specimens since in stained preparations they appear as unformed masses of detritus. They may, in malaria, contain malarial parasites and red cells some of which may contain the parasites and in typhoid fever they enclose many red cells (see Fig. 117,).

In pregnancy *placental cells* (syncytium) swept off in the blood-current " are commonly found " in the mother's blood (Veit).

The *fibrin net-work* often radiating from small masses of platelets seen in fresh blood preparations is of value in diagnosis. The amount of visible fibrin is very large in certain diseases, as pneumonia, acute articular rheumatism, etc.

Counting Red Corpuscles.—The blood-counting apparatus (see Fig. 118), consists of a pipette in which the blood is diluted, a counting chamber by means of which a layer of the suspension of corpuscles of known depth and area is obtained and a special cover-glass to serve as the upper boundary of this layer. This apparatus should be carefully standardized and the necessary corrections always made for we have bought expensive pipettes from good dealers which had an error in calibration amounting to 40% and counting chambers with ruling definitely inaccurate. The pipette is a graduated capillary tube (Fig. 118) *A*, opening into a dilation, *B*, at the opposite pole of which is a second shorter glass tube, *D*, to which is attached a rubber tube with a mouth-piece. The pipette is so graduated that the capacity of the reservoir measured from the line marked 1 to that on the shorter tube marked 101, is exactly 100 times the capacity of the capillary tube from its point to the line marked 1. As a rule the unit length of the long tube is divided into 10 sections but the only marks of importance are the 1 and the 0.5. We much prefer those pipettes which have on either side of the 0.5 and the 1 marks 2 smaller marks, each indicating the 1/100 length of the tube (see page 519), since then one need not bother to draw the blood just to the main line but using these short lines and calculating the correction can work more rapidly and therefore more accurately. The point of the long tube should not be too sharp since in the quick movements made it will be easily broken. In the bulb is a small ball, *C*, which aids much in mixing the blood and the diluting fluid. The pipette is cleaned by washing it out first with water, then with alcohol and then with ether Air is then sucked through, not blown, until the bulb is visibly clean and the glass ball rolls freely within it.

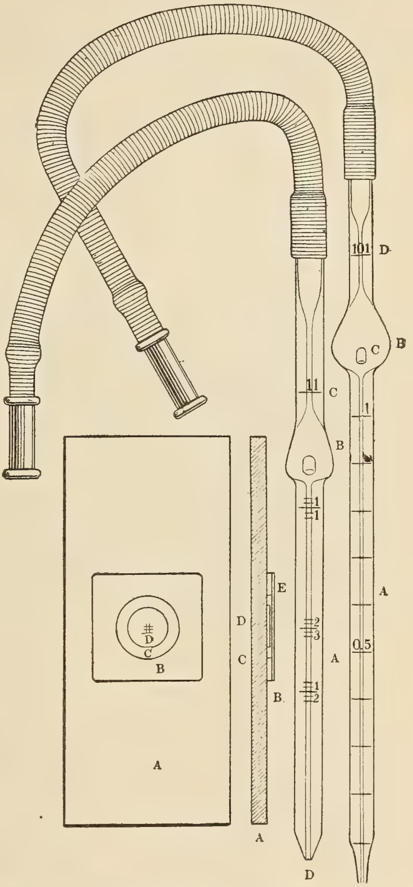

FIG. 118.—Blood-counter (Thoma-Zeiss). To the right is the ordinary form of pipette for red cells; the other is a leucocyte pipette with improved markings and point, D. The ruled counting chamber is shown on edge and face view. A, the slide; B, the ring; D, the ruled table and C, the "ditch;" E, the cover-glass.

Boggs[14] has improved the pipette by inserting in the rubber tube a Wright's "throttle capillary." This consists of a capillary tube which has been heated in a very small flame and then quickly drawn out into a fine thread and an outer, protecting tube from 5 to 7 mm. in diameter and of hour-glass shape. The large part of the capillary is marked with a file, so that it may be conveniently broken off after it has been cemented in the holder. The cementing is easily done by molding a little sealing-wax near the throttled end, passing the larger, free end of the capillary first through the holder, and, after warming gently at the constricted part, drawing the waxed end down into the narrow waist of the tube. The wax softens, fills the neck of the hour-glass tube and on cooling leaves the capillary firmly cemented in place. Each end should be about 5 mm. shorter than the container. The larger end of the capillary is then broken off the point marked, using a pair of fine forceps. When the pipette is washed this controlling device should be removed. This controller makes it easy to draw the column of blood steadily and slowly to the point desired and also keeps the blood from falling from the pipette when the tip is transferred to the bottle of diluting fluid.

Dr. James Wynn has further improved the pipette by providing the rubber tube with a double roller device which makes suction with the mouth unnecessary (Fig. 119).

The apparatus consists of a hair pin (preferably a crimping pin the wire of which is about 0.1 cm. in diameter) and 2 rubber cylinders, shaped with pen-knife from an ordinary Eberhardt Faber " Ruby " eraser and then smoothed with fine sandpaper. The shorter cylinder is approximately 0.8 – 1.25 cm.; the longer is of similar dimension in the center, but about 0.3 cm. longer at either end, these extremities being slightly greater than 0.8 cm. in diameter. This gives the longer cylinder a spool shape and enables its curved surface to exactly approximate the corresponding surface of the other cylinder.

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[14] Jour. A. M. A., January 5, 1907, vol. xlviii, p. 47.

A hat pin is carefully thrust as near as possible through the axis of each cylinder and the 2 are worked onto the arms of the hair pin, reinserting the pin shafts several times in order to enlarge the axis passages until the cylinders rotate fairly easily. The cylinders are then pushed well up on their respective shafts and the curve of the pin is bent so that corresponding surfaces just touch. The pipette tubing is then slipped between the cylindrical rollers. In use the rollers are gripped gently between thumb and index finger, and rolled away from the attached end, compressing the rubber tube between them as they go. The vacuum thus created enables the operator to control with finger tip accuracy the rise of blood in the tube. When the blood mark has been reached the tip of the tube is plunged in the diluting fluid and enough drawn in to empty the capillary of blood. The rubber tube is then released from between the rollers and using mouth suction the glass bulb almost filled. (This enables one to rotate the bulb while filling it etc.). The tube is then gripped by the rollers as before and the diluent graduation is reached promptly and exactly with no danger of overflow into the rubber tube.

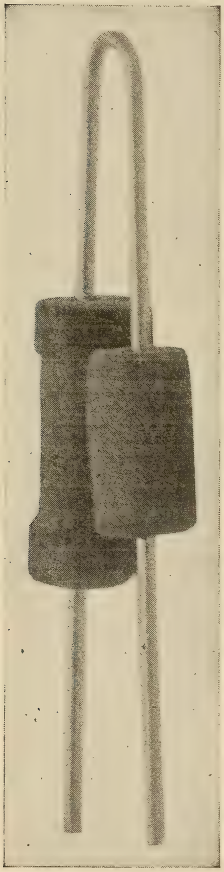

FIG. 119.—Wynn's roller device for rubber tube of mixing pipette.

The worker will save much time if he pays careful attention to his pipette. It should be thoroughly clear before it is used, the capillary should contain no trace of clotted blood, the glass ball should roll freely in the bulb and the rubber tube should be very flexible, not cracked near the mouth-piece, and should contain no saliva.

Of the *diluting fluids* in use Toisson's is the best. The formula of this is:

Water (distilled),	160 c.c.
Glycerin (neutral),	30 c.c.
Sodium sulphate,	8 gms.
Sodium chloride,	1 gm.
Methyl violet,	0.025 gm., or just enough to give desired tint.

Hayem's fluid is preferred by some:

Distilled water,	200 c.c.
Sodium chloride,	1 gm.
Sodium sulphate,	5 gms.
Mercuric chloride,	0.5 gm.

Sodium chloride can be used in rather strong solution (3%). It is probable that the physiological 0.6% solution will lake a certain number of corpuscles.

These diluting fluids when used must be fresh and recently filtered since yeasts grow in them which lead to error. (We remember one case with normal leucocyte count in which a count of 110,000 cells was reported.)

Some of the *counting chambers* consist of a heavy glass slide, (Fig. 118) *A*, on which is cemented a thick glass ring, *B*, the surface of which is carefully polished. This ring surrounds a circular table of glass, *D*, upon which is the ruled area and the height of which is just 0.1 mm. less than that of the surrounding ring. Between this ruled glass table and the inner edge of the ring is a small ditch or moat, *C*, to catch the drop of diluted blood which may run off from the table and to prevent this from running up between the ring and the cover-glass on the other side of the ditch. The later models (see Fig. 120) are more convenient since they have, instead of the thick glass ring, 2 parallel tables between which is a third just 0.1 mm. lower which is a narrow rectangle the ends of which reach nearer the sides of the slide than do those of the 2 higher tables. Those with open moat have the great advantage that the cover-glass may be accurately adjusted and then the diluted blood allowed to run up beneath it by capillarity. There is, therefore, no danger that the chamber must be cleared up several times before Newton's band's appear.

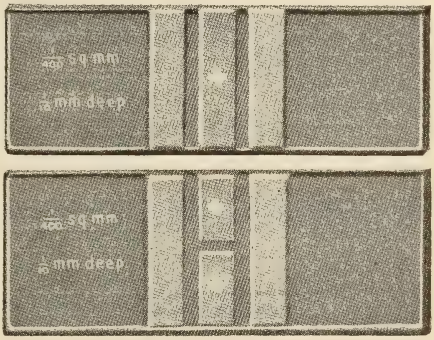

Fig. 120. — Counting chambers with open moat (Buerker). A, with single ruling; B, with double ruling.

On the central glass table of the older model and in the middle of the 2 glass tables of the latter are ruled 21 parallel lines, 0.05 mm. apart. Crossing these at right angles is an exactly similar set of lines. The result of their intersection is a 1 mm. square, divided into 400 equal small squares (see Fig. 121). Through each fifth row of squares is ruled an extra line. This extra line is not a boundary but merely aids the observer to keep his position in the ruled area Indicated, not bounded, by these extra lines, the square millimeter is therefore divided into 16 units of 25 squares each. Other rulings are in use all of which agree in that one square millimeter is divided into many smaller and easily recognized units.

When choosing a blood-counter these lines should be carefully studied since certain makers have put on the market very imperfectly ruled slides. They should first be examined dry to make sure that the lines are complete and then, when covered with a drop of water that their sharpness may be determined; for we have seen lines which appear very distinct on a dry slide practically disappear when covered by a drop of water.

Before use this counting should be washed with water and carefully wiped with a soft cloth (a coarse one will blunt the edges of the lines making them indistinct) care being taken that no lint be left on the surface of the glass ring. Alcohol or ether should never be used for this purpose since they will dissolve the cement which fastens the center glass table.

The cover-glass is a heavy one with planed surfaces made particularly for this use. Ordinary cover-glasses should never be used for their surface may be uneven, they are seldom flat and are so thin that the capillarity of the drop will bend them slightly.

Diluting the Blood.—After the ear or finger has been pricked deeply (see page 428) so that the blood flows freely without the assistance of pressure, a large drop is allowed to collect on the skin and is rapidly drawn into the pipette to the mark 0.5 or 1 according to the condition of the blood. Normal blood should be drawn only to the point 0.5, anemic blood to the point 1. If drawn too far the column may be shaken down somewhat by tapping the point of the tube against a towel or rubbing it against the end of the finger, but unless there is very little correction to be made the instrument would better be cleaned up again and the whole work started anew. It is for this reason that we prefer the special marking mentioned on page 452, and also that devices like those described on page 453 are of value. If the column is of the right length the tip of the pipette is rapidly cleaned, either on the finger or by wiping it on a towel and plunged into a bottle of the diluting fluid which is now drawn up into the pipette until the mixture reaches the line 101. While drawing in this fluid the tube is held vertically

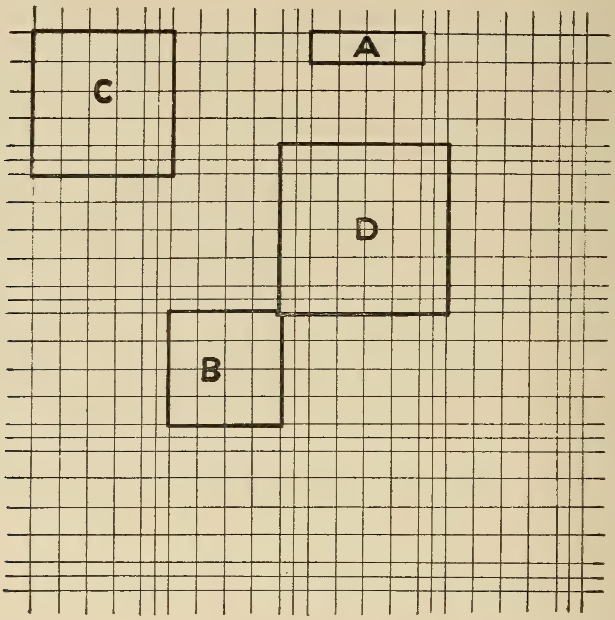

FIG. 121.—The one square millimeter ruled area, much magnified, showing the units in common use.

and rotated between the finger and thumb in order that the diluting fluid may mix at once with the blood as it enters and that no bubble of air may cling to the inside of the bulb. When the fluid reaches the mark 101 (an error of 1 mm. in this case would mean a negligible error of only 0.03%) the pipette is withdrawn from the diluting fluid, its ends closed by the thumb and first finger and it is shaken vigorously in a direction at right angles to its long axis for at least 1 minute. Two or three drops are then blown out in order to empty out all the fluid which has not entered into the mixture. If the blood is not to be counted at once the pipette may be sealed for several days by stretching a wide rubber band over its ends. When the count is to be made the shaking is repeated as vigorously as before.

To fill the counting chamber the pipette is well shaken, the capillary tube emptied by blowing out 2 or 3 drops and a small drop, the size of which can be learned only by practice, is blown out upon the ruled table and covered at once with the cover-glass. Theoretically the drop should be just large enough to cover the ruled glass table and yet none flow over into the ditch, but practically some may run into the ditch but not enough to run up onto the surrounding ring. The cover-glass should be put in position at once. The best way to do this is, we think, to grasp it by 2 diagonal corners, to place a third corner against the slide with the edge of the glass ring as a fulcrum, and then allow it to rotate down onto the drop. In this way no air-bubble is enclosed.

The next step is to determine whether any dust or dirt between the cover-glass and the glass ring is increasing the thickness of the layer of diluted blood. This is done by holding the slide almost on a level with the face and toward the window in such a position that light is totally reflected from the surface of the cover-glass. If the cover and slide are in good apposition a beautiful spectrum band of colors (Newton's bands) should appear over the surface of the glass ring. Should these colors not be seen the cover-glass may be touched by some instrument (but not by a pencil). This may bring out the color bands. If they remain when the pressure is relieved the specimen is satisfactory. If, however, the concentric Newton's rings appear around the point of pressure and disappear when the pressure is removed the slide should be cleared up and another trial made since the increased thickness of the layer of blood will lead to great error in the final result.

This test of good technic, the phenomenon of light interference, is to many a great bugbear but the fault usually lies in the counting chamber itself. We have bought slides on which the bands could almost never be obtained and others using which we seldom failed to get them. In the case of the newer instruments we get the cover in position first and then introduce the blood. While handling the glass slide it should be kept as nearly horizontal as possible since a slight tilting may allow the cover-glass to slide off. The counting chamber should now be allowed to rest for from 3 to 5 minutes in order that the corpuscles may settle onto the surface of the glass and therefore be counted more easily since all are in one plane.

It will be seen that at certain points of this technic the movements must be very rapid. It is no exaggeration to say that greater mistakes are sometimes made by too careful than too quick work. It is of great importance that the pipette capillary be filled quickly, that the dilution be made rapidly (otherwise one finds groups of corpuscles not broken up by the shaking), that no time be lost between the final shaking and the blowing out of the drop into the ruled area and, in the older chambers, that the cover-glass be at once placed in position.

The student who uses a chamber with closed moat will soon learn that it takes less time to clean up his counting chamber or his pipette and begin anew than to count a lot of extra fields with the hope of correcting some error which he is conscious to have made. One saves time by working with 2 counting chambers since the cells on one can settle while the other is being counted.

It is also of great importance to examine the ruled area carefully with the low power before beginning the count in order to be sure that the cells are fairly evenly distributed over the table. If this is not the case the slide should be cleaned up and another preparation made.

In *counting the cells* a medium high dry power of the microscope should be used by beginners, later the lower powers. A mechanical stage is often useful and yet it is better to train the fingers to move the counting chamber.

The unit of the ruled surface (see Fig. 121) to use is a matter of individual preference. Cabot recommends 1 of 36 small squares, *D*; that is, a unit the 4 sides of which are rows of squares through each of which passes 1 of the extra lines. Simon prefers a unit of the 16 squares, *B*, through none of which the extra lines pass. Sahli recommends a unit of 4 squares, *A*. We count the 4 corner units of 25 small squares each of 1 specimen and then clean up the chamber, shake the pipette well, blow out several drops, fill the counting chamber again and count these same units. That is, we count 8 units, or 200 small squares or ½ of a square millimeter. This is the least that a beginner should count. Later he may count the 2 diagonally opposite corner units of 2 preparations, or ¼ of a square millimeter. When counting, those cells which touch even with their edge the upper and the left-hand lines are included in the unit, while those cells which touch in any way the right-hand or the lower boundary lines even though the cell lies entirely inside the square are to be disregarded. Since one counts downward and to the right there is less danger of counting the same cell twice if this rule be followed. The beginner should not try to avoid leucocytes but rather count them as red blood-cells. If in normal bloods all were counted as red cells the error would be but 0.09% which is of course negligible, although a high leucocytosis in a case of anemia would introduce considerable of an error. In counting leukemic blood it is best first to count all leucocytes with the reds and then to count the leucocytes alone (see page 461). The difference will be the red cell count. Many of the red corpuscles will appear distorted and in some anemias the many very small cells are easily overlooked which may explain the very high color index in certain cases of pernicious anaemia.

If the students counts 8 unit quares of 25 small squares each, then the sum of the cells counted multiplied by 2 will be the number of cells in a layer of diluted blood 1 mm. square and ⅒ mm. deep. If the blood was normal this would be about 2250. This multiplied by 10 will give the number of cells in a cubic millimeter of the diluted blood, and this multiplied by 200 (providing the blood was drawn to the 0.5 point) the number of cells in a cubic millimeter of undiluted blood. This is the desired figure. In case, however, any other unit was used the calculation would differ accordingly.

Our students are taught that if the extremes of the 8 counts, each the number of cells in a unit of 25 small squares, differ by over 25 cells they must repeat the entire count using a new blood mixture. If their technic is good it will be easy to fulfill these requirements but if a mistake has been made it is easier to clean up and begin over than to try, by counting more units, to offset an error due to poor distribution.

We require third-year medical students to count the blood of 1 person daily at the same hour until the difference between the counts of 2 successive days is not over 200,000 cells and the difference between the highest and lowest counts of the 8 units for each day not over 25 cells. That is, we allow an error of 4%, which is enough to include any physiological variations in the count and the error due to counting, which should not be over 2%. Some students attain this accuracy quickly. Some students, however, repeat this daily counting for from 20 to 30 or even more days before their work was satisfactory to themselves or to us. By this time they certainly have learned wherein lies the error in their technic. It is of interest that the most careful ones sometimes make the great errors, since they take too much time where speed is essential. Only those who have tested their own accuracy know how inaccurate they can be. One trained in the above manner is seldom guilty of reporting "rises or falls" of 100,000 cells, nor would he ever report a count of e.g. 4,750,600. The student who knows that he can comply with this rule has a justifiable confidence in his technic. Blood-counting requires considerable practice. Even good workers after a vacation of a few weeks find that it is necessary to practice a little before they are ready again for accurate work.

After the count is finished the slide should be washed with water only and dried with a soft rag, and the pipette is rinsed first with water, then with alcohol and then twice with ether. Air is then sucked through it until the glass ball rolls easily. If alcohol is drawn in before the blood is entirely removed an albumin precipitate will form. To remove this the pipette is filled with a pepsin-hydrochloric-acid mixture and left in the thermostat overnight. In case a clot obstructs the bore of the pipette it may be dislodged with a horse-hair. A fine wire should not be used for this will easily crack off the end of the tube.

Trained workers have considered an error of 2% unavoidable and some are satisfied with 1 of 3%, which would mean that 2 men counting with equal accuracy the same normal blood at the same time might get results which differ by about 150,000 cells. We know no better way to stimulate students to attain good technic than by requiring a certain number of them, the more the better, each with a separate instrument to count independently the same blood. We have seen this result in considerable extra practice.

The Hematocrit.—The hematocrit promised at first to save much of the time it takes to count blood since by it we can determine the volume of the red blood-cells, that is, the volume of the hemoglobin-containing protoplasm. This instrument is a centrifuge capable of very high speed. Each arm of the centrifuge (see fig. 122) holds a small glass tube of rather large bore calibrated with 100 divisions. One of these tubes is inserted in a rubber tube with a mouth-piece and the blood drawn in until the tube is even more than full. This requires a very large drop. The finger, covered with vaseline, is then placed over the free end and then the rubber tube removed. This glass tube is fastened in one arm of the centrifuge and in the other is placed an empty tube to balance the machine. The machine is then revolved at as high a speed as possible until the column of centrifugalized corpuscles does not decrease. Each division of the tube corresponds approximately to 100,000 cells. As a means of counting normal blood this method is fairly accurate but it is the abnormal bloods which it is important to examine and in these the variations in the size of the corpuscles introduces too great an error to be overlooked. But the instrument has its uses. By means of it we may determine the volume index of the red cells, the numerator of which is determined with this instrument (the count is the denominator). Capps[15] certainly has published some interesting results. By means of it we also may examine the plasma for bilirubinemia and for lipemia.

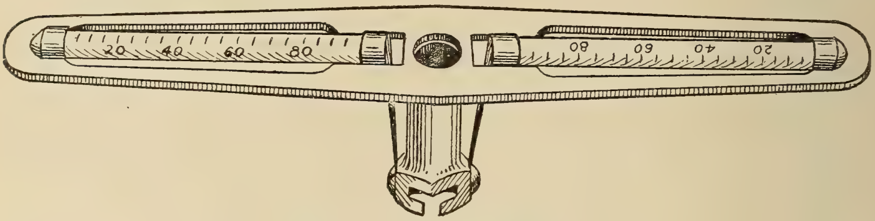

Fig. 122.—Arm of hematocrit.

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[15] Jour. of Med. Research, 1903, vol. vi.

Leucocyte Counting.—The leucocytes may be counted in the same preparations with the red cells, especially if Toisson's fluid was the diluent used. That is, after counting the red blood-cells one counts the leucocytes over the entire square millimeter. The trained eye will pick the most of them out, more because of the difference in their refractivity than from their stain, since they appear brighter when the focus is slightly raised. On the Thoma-ruled slide the leucocytes on the entire millimeter field of 8 separate drops should be counted. This requires considerable time and the number of cells counted is much too small, yet a fairly approximate result is obtained. It is much better to lake all the red cells by using as diluent 1 % acetic acid and to use the same pipette as for the red count but to draw the blood to the 1 line (giving a 1 : 100 dilution) or to use special pipettes which will give a dilution of 1 : 10 or 1 : 40 (see Fig. 118). The fluid is made up by mixing 1 c.c. of glacial acetic acid and 99 c.c. of distilled water. The 0.3% solution mentioned in several text-books is hardly strong enough since the red blood-cells will not be entirely laked and the groups of shadows left are confusing. This mixture should be made up fresh each day, for yeast-cells which resemble mononuclear leucocytes will grow in the dilute acid. It requires considerable practice to use these big pipettes. Their bore is so large that the blood easily drips out; it is difficult to wash the blood entirely into the bulb by means of the acetic acid; and in shaking it is easy to shake the leucocytes down into the fluid filling the tube. To reduce these errors as much as possible the pipette should be held almost horizontal while the blood and then the acetic acid are drawn into the tube. For this reason the bottle holding the acetic acid should have a wide mouth. The acid should be sucked in rapidly that the stream may wash the tube well. The pipette, with its ends firmly closed, is then shaken in all directions except in that of its long axis. In this case also the specimen should be first inspected with the low power to make sure that the distribution of cells is even.

If the counting slide has the Thoma ruling and but 1 sq. mm. area ruled and if the dilution is 1 : 100, at least 8 different slides should be prepared and counted; 5, if the dilution is 1 : 40. If however the ruling gives 9 sq. mm. for the count (Fig. 123) then 3 specimens should be counted. At least 100 lenucocytes should actually be counted in each blood examination made and more if possible. If the acetic acid is of proper strength, is fresh and if the pipette is clean all cells seen may be counted as leucocytes. If the total number of cells counted be divided by the number of 1 sq. mm. units examined, this quotient multiplied by 10 and this by the dilution, the product will give the number of leucocytes in 1 c.mm. of undiluted blood. If nucleated reds are present in the specimen their nuclei will be counted in the acetic acid as small mononuclears. It will, therefore, be necessary to determine their number relative to the number of leucocytes by the differential count of a stained specimen and then make the proper correction of the leucocyte count. The hour the blood was taken for a leucocyte count should always be stated, the temperature of the patient at that time and whether or not the patient had partaken of a proteid meal.

The student should be required to test his own accuracy. The error in leucocyte counting with standardized instruments is usually at least 5%. If a large number of leucocytes is counted it may be reduced to about 3.5%. We are sure that the error made by the busy ward man is nearer 20% and yet we hear, for instance, of "a rise of leucocytes from 10,000 to 11,000 per c.mm.," etc. A careful man will by repeated controls make sure that the error of his technic is not over 5%. This can be done by filling several pipettes at the same time and counting them separately; or better, by inviting another in whose work he has confidence to make a series of parallel counts with him. In control work the blood should be taken at the same time and from the same incision, for one can obtain curious results if he takes the blood from different parts of the body, especially if he uses the ear on which the patient has been lying or the hand which has been in a hanging position.

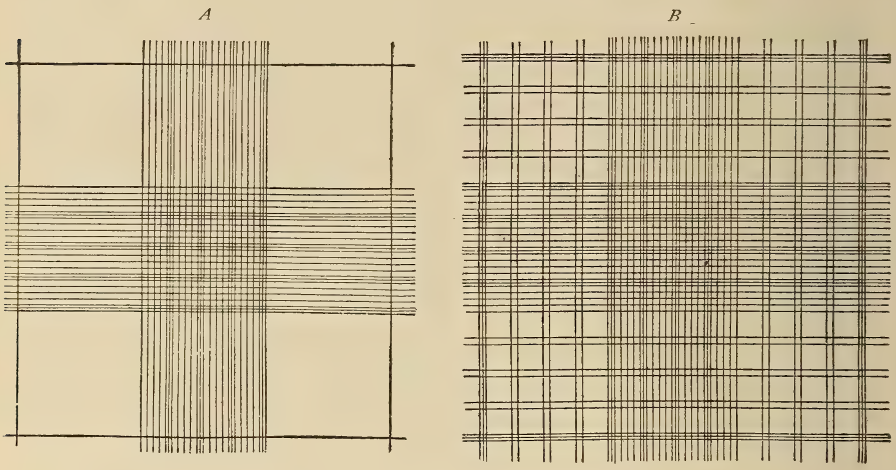

FIG. 123.—*A*, Zappert ruling; *B*, Türk's ruling.

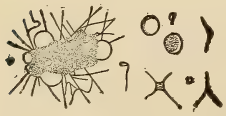

FIG. 125.—Platelets (copied from Osler): *a*, platelets in irregular shapes; *b*, with clear areas; "*c*," Schultze's granular mass.

Blood Smears.—To get satisfactory stained specimens one must first get thin, well spread smears. The best method is that Ehrlich recommended. Two cover-glasses, ¾ of an inch square and of the thinnest glass, are thoroughly cleaned in alcohol and ether (see page 428) and then dried. One cover-glass is held along one entire edge by the crossed-bladed forceps. The other cover-glass is placed in a convenient position to be quickly picked up with the pinch forceps. A small drop of blood about the size of a small bead (about 1.5 mm. in diameter) is

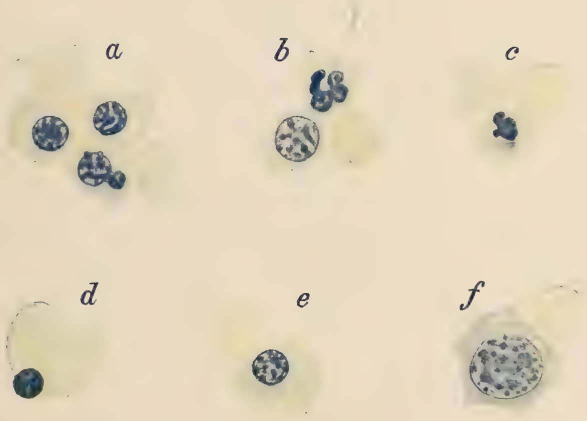

FIG. 124.—Nucleated reds from the blood of a fetus 15 cm. long. *a*, mature nucleated red; *b*, intermediate form and rosette; *c*, mature red, nucleus fragmented; *d*, free nucleus of a mature red; *e*, mature red, polychromatophilic cell; *f*, polychromatophilic megaloblast.

picked up on the last mentioned cover-glass which is then at once dropped onto the other cover-glass. If the covers have been properly cleaned the blood will spread rapidly without the assistance of any pressure other than the weight of the upper glass. Just as the film is about to stop spreading, but before it has stopped the 2 covers are pulled apart in the direction of their planes by a steady but quick motion (see Fig. 105) which requires a little practice. Beginners may find it easier to hold the free cover-glass in the fingers but the moisture from the skin injures the specimens to a slight degree. With the 2 pairs of forceps one can make 100 or more smears in less than 15 minutes. As soon as the covers are drawn apart they are allowed to dry in the air, not over a flame. They then remain spread out on a sheet of paper, blood side up, for from 15 to 30 minutes to become perfectly dry, but must be guarded against flies which suck up the hemoglobin, making large holes in the specimens. For some stains the smear is not allowed to dry but is dropped at once into the fixing fluid. Dry smears should be guarded from dust and moisture.

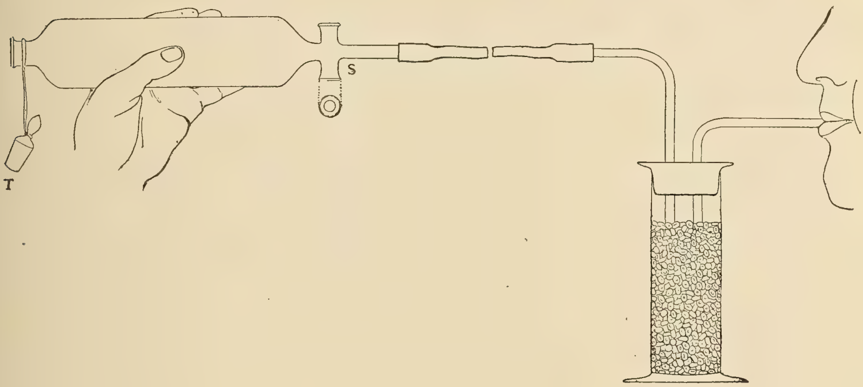

FIG 126.—Showing operator saturating blood plasma with carbon dioxide.

Some prefer to use slides instead of cover-glasses. A large drop of blood is picked up on a slide and is spread at once by the edge of another slide, the ground edge of a glass spreader, the edge of a strip of paper or by drawing a needle flat across the slide. The use of slides has two advantages—no cover-glasses need be used, and a much larger blood surface is obtained for study. On the other hand only a few smears can be made at a time; more blood must be used; and the slides are bulky. But the most important objection is that the spreads cannot be as even. The leucocytes do not spread as do the red cells, but stick to the glass, some forms more quickly than others. In the areas too thick to study one may be sure there will be too many white cells relative to the red cells and relatively too many of some forms of leucocytes. Several smears have been sent us as illustrations of extreme leucopenia. In one case it was claimed that not a single leucocyte could be found, but further study showed many in the thick areas. Masses of leucocytes may be found on the areas first covered by the drop and give there the picture of an extreme leucocytosis. Even when 2 cover-glasses are used the picture is not just the same on both. One cannot assume that the percentage relations seen in the thin areas is true of the thicker areas. We feel that one reason why differential counting has yielded such meager clinical results is the use of slides and the consequent inaccuracies of the results. Even with the most careful technic many of the most interesting cells are usually ruined. We refer to the macrophages and to the very large mononuclear cells which can be found in fresh specimens and in sections of drops of blood hardened en masse.

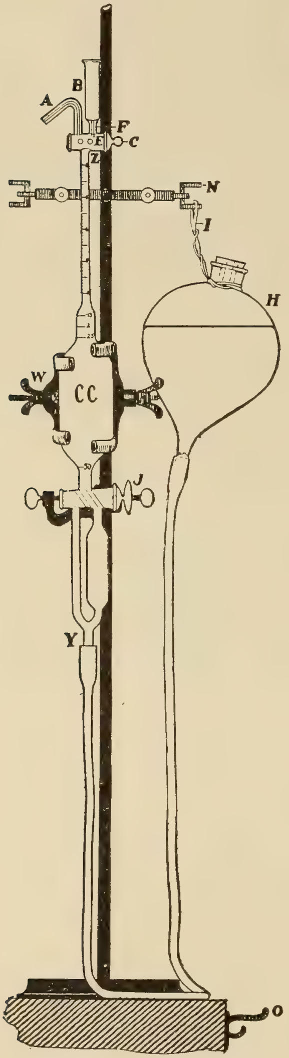

Fig. 127.—CO_2 apparatus.

Air dried smears may be kept for some time if Ehrlich's stain is to be used but for the methylene blue-eosin mixtures now in vogue it is much netter to stain the smears at once even before they are quite air-dry.

Fixing Methods.—The fixing methods used will depend upon the stain to be employed. Among the various methods are:

Methyl alcohol is now the most popular fixing agent, since it can be used as the solvent of many stains and so allows fixing and staining to be simultaneous. With other stains ethyl alcohol is used.

Heat.—This method, the most difficult of all to use well, is the only one which gives satisfactory results with Ehrlich's triple stain. This is best done on a large triangle of unpolished copper plate, with a gas burner under the point. This is allowed to heat until at a constant temperature and then the boiling point is determined with drops of water. The cover-glass is placed on the copper plate with its outer margin (the margin farthest from the flame) ¾ of an inch, blood side up, inside of the boiling point. Here the temperature is from 110° to 115° C. This point can be determined more accurately by dropping toluol or xylol on to the plate since this is their boiling point. The smears should be left at this temperature for an hour and a half to 2 hours. If the blood is heated on the day when the specimen is made 2 hours of heating will be hardly long enough. Specimens a week old generally require an hour and a half of heating and still older specimens less than an hour. The duration of heating depends also on the patient's disease. Normal blood requires the longest heating. Blood from a patient with splenomyelogenous leukemia is usually ruined if left on the bar more than 1 hour and to heat the blood of a patient with pernicious anemia a few minutes too long will often spoil the specimens. We place 4 smears on the bar at the point described above and remove the first in 1 hour and the others later at intervals of about 20 minutes. One of these is almost certain to stain satisfactorily.

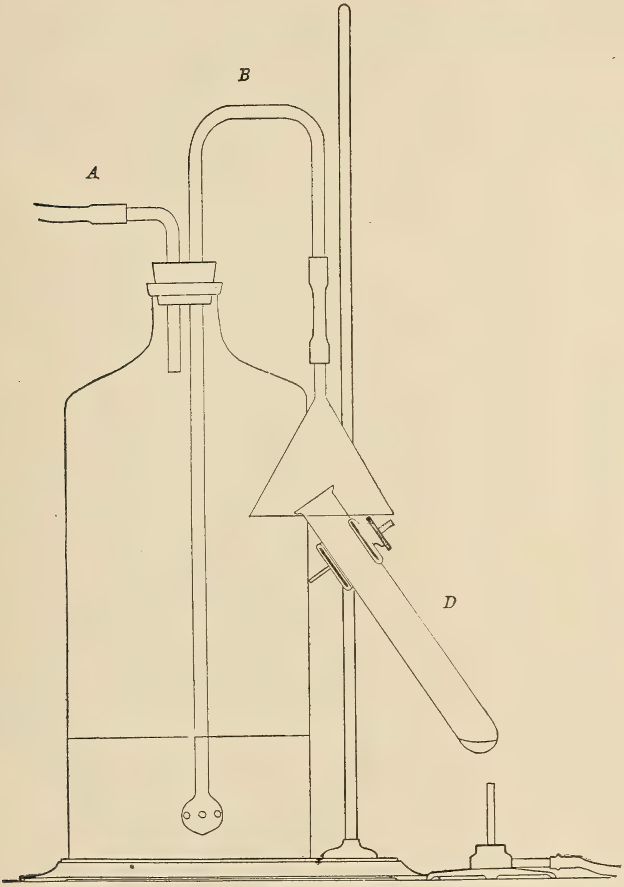

FIG. 128.—Apparatus for removing fumes in connection with nitrogen determinations.

Others place the smears, blood side up, for from half a minute to 2 minutes on the spheroidal point on the bar; that is, at the point where the drop of water just rolls off without boiling. Here the temperature is from 140° to 150° C.

Success with Ehrlich's stain will depend in large measure on the heating; an over-stained smear is underheated and vice versa. The red cells are the best index of success. They must take an orange color with no fuchsin tint and yet not a lemon-yellow tint.

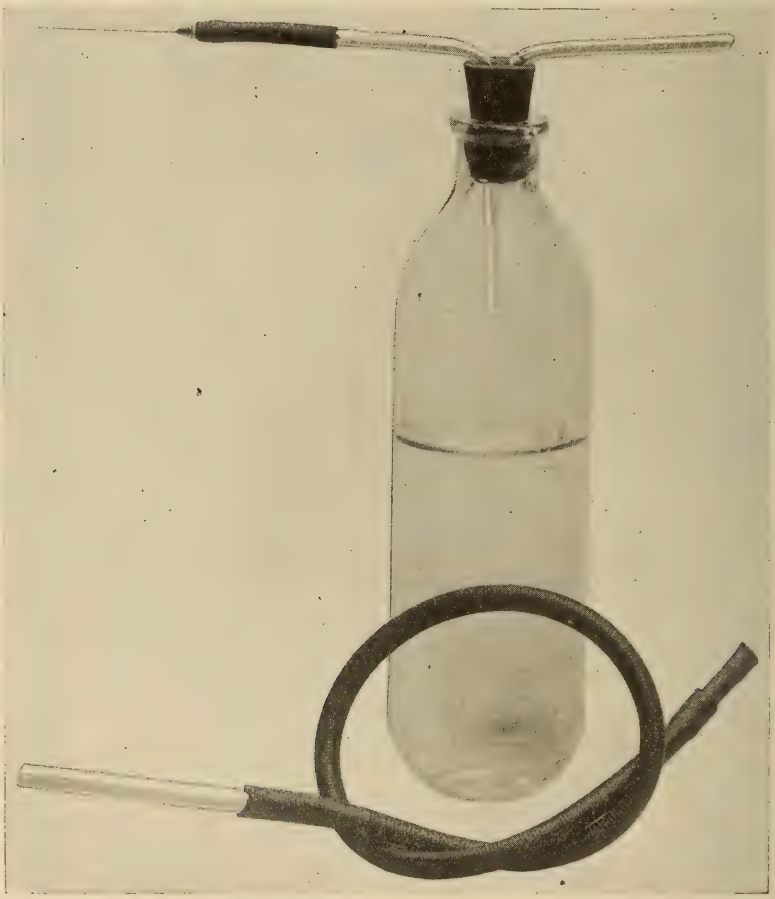

FIG. 129.—Apparatus for Blood Cultures.

STAINS.—The stains were classified by Ehrlich as acid, basic, and neutral, not according to their chemical reaction but according to the nucleus of the stain which serves as the dye.

The classical illustration of this is the following: ammonium picrate is an acid stain since it is the picric acid and not the ammonia which is the dye; rosanilin acetate is a basic stain, since it is the basic element which is efficient, not the acetic acid. Rosanilin picrate would be a good illustration of a neutral stain since both the basic and the acid nuclei would stain. As a matter of fact the neutral stains are all mixtures of 1 or more stains and it is very hard to state just how the compound arises and what it is.

Among the basic stains may be mentioned methyl green, methylene blue, fuchsin, methyl violet, Bismarck brown, and saffranin.

Among the acid stains, eosin, aurantia, the salts of picric acid, indulin, acid fuchsin, orange *G*, and a long list of others.

Neutral stains arise in mixtures of the above. For instance, mixtures of fuchsin and methyl green; of methylene blue and eosin.

(1) *Hematoxylin Eosin.*—This stain is not used nearly enough, since it, best of all, brings out the nuclei of the blood-cells.

Mayer's Solution.—This stain contains hematoxylin 1 gm., alcohol 100 c.c., alum 50 gms. added while cool and then 1000 c.c. of boiling distilled water. A few crystals of thymol are then added and the whole cooled and filtered. It must be kept in the dark. One must determine by experiments how long to stain the specimens, after which they are washed rapidly in water. The nuclei alone will take the color. Eosin, 0.5% aqueous solution, may then be added until the red blood-cells are just rose red. The specimen is then washed in water, dried and mounted. The protoplasm and the nuclei are beautifully stained but not all the granules.

The polychrome methylene blue-eosin stains are at present practically the only ones in use. They are the only stains which bring out the chromatin of the malaria parasite; they stain the nuclei of the leucocytes very well and also the Mastzell granulation. The eosinophile granules are well stained but not so the neutrophile although perhaps as well as is necessary. If, however, one is studying granulations especially he will not use this stain alone, nor, indeed, any stain containing methylene blue, because it is so tricky. At least 16 different methods [16] of making this stain have been reported, all of them modifications of the original Romanowski's stain.

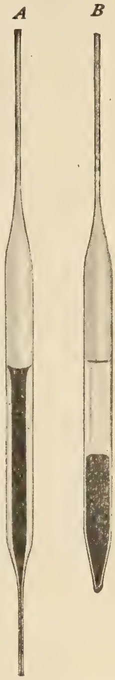

Fig. 130.—Tubes filled with clotting and clotted blood. *A*, blood is clotting spontaneously, the clot now retracting from the sides. *B*, clot in centrifugalized tube.

Hastings' Mixture.[17]—The dry stains necessary are eosin, soluble in water, yellow (Grübler); and methylene blue (Ehrlich's rectif.) (Grübler).

Solution *A* = eosin 1% aqueous.

Solution *B* = alkaline methylene blue 1% aqueous.

Solution *C* = methylene blue 1% aqueous.

Solution *A* will keep, but solutions *B* and *C* must be made fresh.

To prepare *B* add to a warm 1% solution of dry powdered sodium carbonate 1% of methylene blue powder and heat over a water-bath for

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[16] Baumgarten, American Med., 1904, vol. vii, p. 14.

[17] See, also Wright's method, Jour. Med. Research, 1902, vol. ii. ,p. 139.

15 minutes. Add 30 c.c. of water for each 100 c.c. of original fluid, and heat again for 15 minutes. The solution is then decanted from the residue and divided into 2 equal parts. The 1 part is made faintly acid with 12.5% acetic acid (this is best determined by placing a drop on blue litmus paper and taking as the end reaction the point at which the margin of the drop after absorption in the paper shows a faint pink) and then mixed with the remaining unneutralized portion.

To make up the stain mix distilled water 1000 c.c., solution *A* 100 c.c., solution *B* 200 c.c. and solution *C* 70 to 80 c.c. In adding solution *C*, pour in 70 c.c. at once, stir well and if no precipitate is present add more, a cubic centimeter at a time, until one just appears. The stain is then allowed to stand for half an hour and then filtered through 1 filter. Forced filtration is usually necessary. The dry residue is removed from the paper and reduced to a powder in which form it may be kept. Seven to nine-tenths of a gram of dry stain is usually obtained. If more than 0.9 gm. the resulting stain is useless. The staining solution is made by dissolving 0.3 gm. of the dry stain in 100 c.c. of Merck's pure methyl alcohol. To do this the stain must be rubbed up with the alcohol in a mortar and pestle since it is with difficulty soluble.

Usually the blood smear is covered with 2 drops of the stain for 1 minute, which will fix the specimen, and then 4 drops of distilled water are added and the dilute stain left on the smear for 4 minutes. For uniformity, a dropper should be used. These figures are merely examples. For each new lot of stain one must determine the relative proportions of stain and water to be used in staining and the relative lengths of time to let the undiluted and the diluted stain act.

Wilson's Stain.—One makes a 1% solution of methylene-blue in an 0.5% aqueous solution of sodium carbonate and adds at least 0.5% of freshly precipitated silver oxide.[18] The methylene-blue solution is boiled and at the end of 20 minutes ⅓ of it removed. After 20 minutes more boiling ½ of the liquid is removed and the remainder boiled for 20 minutes. These 3 portions of fluid are combined and the mixture made equal to the original volume with distilled water, discarding the precipitate which sticks to the bottom of the evaporating dish. The methylene-blue solution is mixed with an equal volume of a 0.5% aqueous solution of eosin and allowed to stand for 1 hour. The precipitate is collected on a "hard" filter paper, washed with distilled water or preferably with 0.85% sodium chloride, dried and preserved in a dark glass bottle.

To prepare the staining mixture 400 mgms. of the powdered stain are dissolved in 100 c.c. of absolute methyl alcohol. Since the powder is only slightly soluble the solution is facilitated by rubbing it up with the alcohol in a mortar. The stain should be kept in a tightly-stoppered dark glass bottle.

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[18] To prepare the silver oxide, dissolve 2 gms. of $AgNO_3$ in 15 c.c. of distilled water and add about 260 c.c. of milk of lime. Shake well and set aside. Decant the supernatant fluid, collect the precipitate on a filter, wash it out with about 20 c. c. of distilled water, dry at a temperature not exceeding 100° c. and preserve it in a tightly-stoppered dark bottle.

The cheaper grades of methylene blue are said to make satisfactory stains.

If the stain used is one in which the cover-glasses are to be completely immersed much time may be saved by using the holder which Pepper has invented. This allows 55 slips[19] to be stained simultaneously in 35 c.c. of the staining fluid.

For basophilic granules the methylene blue stains, carbol-thionin, or dahlia may be used.

A good carbol-thionin mixture is: thionin 0.3 gm., absolute alcohol 10 c.c. and carbolic acid, 1%, 100 c.c. The smear is stained in this for 2 minutes, washed in water and dried.

A Specific Stain for Endothelial Leucocytes.—Several stains supposed to differentiate between lymphocytes and lymphoid cells or lymphocytes and endothelial leucocytes have been published, among them one proposed by McJunkin and Charlton [20] which brings out a granulation which they consider characteristic of the endothelial leucocytes.

To prepare the solution one adds 0.2 gm. of alphanaphthol (Merck reagent), 0.015 gm. of methyl violet 5B (Grübler) and 0.2 c.c. of hydrogen peroxide to 100 c.c. of warm 80% alcohol (made from absolute alcohol). The hydrogen peroxide used should contain approximately 3% by weight of the gas as determined by titration with decinormal potassium permanganate.

The blood film on a 22 mm. square cover-glass is covered for ½ minute with 5 drops of the above solution to fix the preparation. Five drops of distilled water are then added and the dilute stain allowed to act for 5 minutes. The smear is then washed with water, dried with filter paper, counterstained for 2 or 3 minutes with 0.01% basic fuchsin (Grübler), washed, dried in the air and mounted in balsam.

In specimens thus stained nuclei and cytoplasm are colored red while the cytoplasmic granules in neutrophils and endothelial leucocytes are blue. The granules of basophils take a distinctive red color. The central portion of the eosinophile granules is unstained so that these granules have a very characteristic ring-like appearance. The platelets take the red stain faintly. The erythrocytes are pink.

Since the red cytoplasm of lymphocytes is entirely free from bluish granules the only differentiation requiring discussion is that between the neutrophils and endothelial leucocytes. The granules of the endothelial leucocytes are discrete and the cytoplasm is distinctly seen between them, while the neutrophilic granules are so thickly placed that little of the cytoplasm can be seen. The neutrophilic granules are larger and more regular in shape than those of the endothelial leucocytes. In pathologic blood in which mononuclear myeloblastic cells (neutrophilic myelocytes) are present the character of the granules assumes a greater significance. However, since the granules of neutrophilic myelocytes and of the so-called metamyelocytes are even more prominent than are those in the polymorphonuclear neutrophils, there is no chance of confusing myelocytes and endothelial leucocytes, although both are mononuclear. The differential character of the endothelial leucocyte is the presence of blue granules in a mononuclear cell. Although the nucleus of this leucocyte frequently has a broken outline it does not consist of pyknotic nuclear masses connected by filaments. The reason that endothelial leukocytes cannot be identified in films stained with a polychrome blood stain is that some of these cells are entirely devoid of granulation and cannot, therefore, be distinguished from lymphocytes when they approach these cells in size.

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[19] Jour. of A. M. A., Jan. 11, 1908, vol. 1, p. 122.
[20] Arch. of Int. Med. Aug., 1918, xxii, p. 157.

A Polychrome Stain for Protozoa.—McJunkin's modification of Giemsa's stain.[21] This stain is a single solution of polychrome methylene blue, methylene blue and eosin.

The polychrome methylene blue solution is made by measuring accurately from a buret 50 c.c. of a decinormal solution of sodium carbonate into a 500 c.c. beaker. (This is standardized by titration against a standard acid using a 1% alcoholic solution of methyl red as indicator.) To the carbonate solution is added 1 gm. of methylene blue (Grübler's B. X.) and 50 c.c. of glycerin (Merck U. S. P.) measured in a 100 c.c. graduate. The beaker is placed in a water bath and its contents stirred by a mechanical stirrer which is run at the rate of about 200 revolutions a minute. The solution is kept at a temperature of from 87° to 89° C. for 1 hour. The temperature of the water in the bath outside the beaker should be from 94° to 96° C. At the end of the hour the beaker is removed from the water bath and its contents while still warm poured into a 100 c.c. graduate. The beaker is washed out with 5 c.c. of distilled water to recover any carbonate which may have precipitated out and this is added to the contents of the graduate.

Into a second graduate is measured enough methyl alcohol (Merck's reagent or Kahlbaum's acetone free) to make the total volume of stain 100 c.c. This methyl alcohol is now poured from the second graduate into a 4-ounce bottle and 0.75 gm. of methylene blue (Grübler's B. X.) and 0.25 gm. of eosin (Grübler's yellowish water soluble) added. The bottle is shaken to secure solution of the methylene blue and the eosin in the alcohol. After the dyes are completely dissolved in the methyl alcohol the polychrome methylene blue solution is poured from the graduate into the bottle. The volume of dye is now just 100 c.c. The weights of the dyes must be accurate and the glassware free from acid.

Since some samples of eosin (Grübler's yellowish, water soluble) stain the red blood corpuscles a blue that cannot be washed out with water, one should use an eosin that has previously been found satisfactory in making polychrome staining solutions.

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[21] Jour. of A. M. A., Dec. 18, 1915, vol. lxv, p. 2164.

To demonstrate protozoa and bacteria the smear preparations, which may be made on either slides or cover-glasses, may best be fixed in equal parts of absolute alcohol and ether for from 10 minutes to a number of hours.

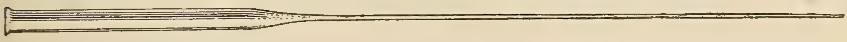

FIG. 131.—Tube used for diluting serum.

Distilled water is placed in a staining dish and to this is added 1 drop of the stain per cubic centimeter of the water. Cover-glass preparations are floated on the solution as soon as the dilution is made and stained for from 30 to 60 minutes. Trypanosomes are well stained at the end of half an hour while about 1 hour is required to stain Spirochæta pallida heavily. The preparation is removed from the stain and an excess of blue is washed out with distilled water until the red blood corpuscles are pink in color. After washing, the preparation is dried in the air and mounted in acid-free balsam. Slide preparations are best stained in an oblong Petri dish without cover (a dish the length and width of a slide and about 2 cm. high), inverted, with one end resting on the end of the dish. In this position the specimen will be as near the surface of the stain as possible. After staining, the slides are washed and dried in the air. They may be examined directly with oil, or they may be mounted in acid-free balsam.

FIG. 132.—Widal test. Field of motile organisms. × 900.

Pappenheim's solution of pyronin and methyl green is composed of saturated aqueous solution of methyl green, 3 to 4 parts, and saturated aqueous solution of pyronin, 1 to 1½ parts. This stain, which may be used as a routine bacterial stain, is useful also in blood work and for distinguishing the nucleus of the erythroblasts from its basophilic granules. The nucleus and all nuclear fragments stain a beautiful blue and the basophilic granules a brilliant red (Morris). The blood spreads should be fixed by heat (Ehrlich's Method).

Ehrlich's Triple Stain.—The words Ehrlich's "triacid" and Ehrlich's "triple" stain are often wrongly used as synonyms. The triacid stain, a mixture of equal parts of the saturated solutions of indulin, nigrosin, and aurantia, was intended to bring out especially the eosinophile granules. It is hard to make up and is now very little used.

Ehrlich's triple-stain is a mixture of the saturated aqueous solutions of methyl green 00, acid fuchsin, and orange *G*. (Grübler's stains are usually used.) The best formula for this stain is that published by Morris.[22]

	c.c.
Saturated aqueous solution of orange *G*	13.0
Saturated aqueous solution of acid fuchsin	7.0
Distilled water	15.0
Absolute alcohol	15.0
Saturated aqueous solution methyl green	17.5
Absolute alcohol	10.0
Glycerin	10.0

These fluids are measured in the same graduated cylinder, which should not be rinsed out during this procedure and are poured in the order given in the formula into a receiving flask which should be shaken vigorously after the addition of each. The methyl green, the second portion of alcohol and the glycerin should be added drop by drop and the flask frequently shaken. The mixture can be used at once. It seems to improve for a time on standing, but later spoils. It should never be filtered and the bottle containing it should never be shaken. The blood smear is covered for from 3 to 20 minutes with a few drops of the stain obtained on a glass rod from as near the center of the bottle as possible. It is very difficult to overstain and films suggesting this usually are underheated. The smear is next washed in distilled water, quickly blotted and mounted in balsam. If washed quickly in absolute alcohol the granules will stand out more clearly, but the nuclei will be paler. In a successfully stained specimen the red blood-cells will have a buff color without the slightest shade of red, the nuclei of the leucocytes will be a dark green and those of the normoblasts almost black, the fine granules will take a lilac stain and the coarse a crimson. This is the only stain which gives a specific color to the fine granules. It is for this reason that it was introduced. It is inferior to other stains for protoplasm and nuclei and does not in the least stain the Mastzell granulation. If one desires to get a good idea of the blood as a whole, other

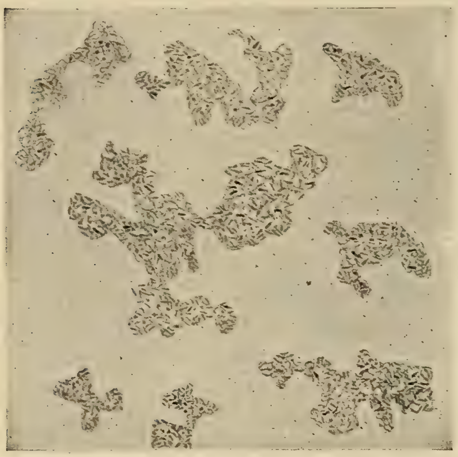

FIG. 133.—Widal test. Field of agglutinated organisms. × 900.

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[22] Jour. of A. M. A., Aug. 6, 1910, vol. lv, p. 501.

stains a so should be used, preferably hematoxylin and eosin, or methylene blue and eosin, etc.

The blood of some persons takes the Ehrlich stain poorly, while that of others takes it well. In certain diseases, particularly lymphatic leukemia, it is almost impossible to get a good specimen with Ehrlich's stain because the basic element is so markedly lacking.

Ehrlich's dahlia stain consists of distilled water 100 c.c., saturated alcoholic (absolute) dahlia solution 50 c.c. and then, on clearing, 10 to 12.5 c.c. of glacial acetic acid. The specimen, heated or fixed by alcohol, etc., is stained in this solution for from 5 to 10 minutes.

ERYTHROCYTES

The red-blood corpuscles are specialized non-nucleated cells which consist of hemoglobin, 95%, and of stroma. Their chief function is to carry oxygen to the tissues and, to a lesser degree, carbon oxide to the lungs.

In SHAPE they are circular, discoid cells, which in the circulation may be cap-shaped (Weidenenreich and Lewis) but which in well-made fresh preparation lie flat. In many, but not in all normal cells, a biconcavity is apparent but in the secondary anemias especially this is very evident. These cells of the normal blood, unless subjected to considerable mechanical injury, are perfectly round and in size vary from 6 to 9μ in diameter. Those which are not round are called "poikilocytes" (Plate I, 25–28) Such cells occur in pernicious anemia especially, even in cases of a mild grade and in other anemias of very severe grades especially those due to cancer, tuberculosis, etc. They are said to be the results of alterations in the plasma, but may also be "badly made" cells.

STRUCTURE.—Red-blood cells are so sensitive that they are about the hardest of all cells to study. When fresh they certainly look structureless but when stained each method used has indicated a different structure. The concensus of opinion now is that all of the fibers, layers, etc., described in these cells are artefacts; that the various granule-like bodies seen in the fresh cells are not an essential part of the cell and that those seen in stained cells are in part, at least, precipitates of the fixing agent or of the stain; and that any definite structure, although it certainly must exist, is yet to be demonstrated. Ehrlich's argument that heat must be the best fixative agent because it gives homogeneous cells may be the best argument against heat.

Not only their fine but also their coarse structure is in dispute. A true cell membrane has never been proven although none doubt that the peripheral layer of the cell does serve the function of a cell membrane, whether it is a membrane or merely a concentration of the stroma at the surface,[23] or a layer of hemoglobin-stroma in a slightly different physical condition. Some still insist that these cells have a true membrane (see page 662).

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[23] See Peskind, Am. Jour. Med. Sci., 1904, vol. cxxiv.

Those who believe that the nuclei of these cells disappear within the cells think it necessary to find some remains of it there. The "nucleoid," so often mentioned, is still in dispute; some considering it to be related to the nucleus and others to be totally independent.[24] This, also called the "differentiated inner body of Löwit," is a nucleus-like structure which in stained specimens is very apparent in the center of many of those red-blood cells which take a basic stain. It has a fibrillar structure and a central clear space. It may contain an inner body which "may be extruded as a platelet." "This nucleoid develops after the extrusion of the nucleus" said Maximow but Löwit considered it the remains of the now invisible nucleus. Whether the erythrocytes in the normal circulations are to be considered as living or dead cells is a question which recently has attracted considerable attention. This is largely a question of definition. They are very sensitive to injury and degenerate very rapidly when removed from the blood. Only perfect cells are seen in the circulation. They enter it as such and they leave it before the signs of age are apparent (see page 507).

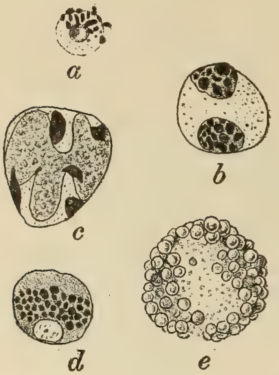

FIG. 134.—*a, b, c, d,* four leucocytes containing Löwit's organisms (copied from Löwit); *e,* large granular (and vacuolated?) cell of bone-marrow.

SIZE.—The red cells of the adult vary from 6 to 9μ (average 7.5μ) in diameter. Hayem found that 75% varied from 6.6μ to 8μ; 12.5% from 6μ to 6.6μ, and 12.5% from 8 to 9μ in diameter. In the normal adult these cells are fairly uniform in size, although a very few dwarf cells are found at all ages. In the normal infant's blood, however, these cells vary much more in size, their limits being from 3.3μ to 10.3μ. In disease the adult type of blood may assume this infantile condition. There is evidence that the red-blood cells of various nationalities differ somewhat, their size diminishing as one approaches the equator. In the fresh blood certain physiological rhythmical changes in size may be noted, the cells becoming somewhat larger in the venous than in the arterial blood (Hamburger). Pathologically, they vary much in size.

Microcytes.—By microcytes is meant a cell under 6μ in diameter. The most measure about 3.5μ and yet some are 2.2μ in size. Not all of these small cells are schistocytes, *i.e.*, fragments of larger cells (although one can watch the process of constriction of small fragments from red blood-cells in fresh blood subjected to mechanical or chemical injury) for microcytes are seen in perfect fresh specimens and we find nucleated microblasts 3.5μ in diameter which must represent young forms of microcytes. Microcytes are not biconcave as a rule but are spherical and hence have a deep color. They occur normally in large numbers in the embryo infant and especially in the blood of premature children in which cases they are often polychromatophilic. They are found rarely in the healthy adult, but are common in all anemias, especially the primary and severe secondary anemias.

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[24] Maximow, Arch. f. Anat. u. Physoil, 1899.

Macrocyte is a term applied to cells from 9 to 12μ and above in diameter; for cells from 12 to 16μ the term *megalocyte* is used, and for those above 16μ *gigantocyte*. These cells occur in largest numbers in pernicious anemia. Some believe that if 10% of the red cells of any given case are macrocytes a diagnosis of pernicious anemia is justified. They also occur in leukemia and in chlorosis. In chlorosis they are often very pale, hence are termed " chlorotic " or " dropsical " cells. They are common in cases with cholemia, which is of interest since patients with pernicious anemia are so often jaundiced (Osler). Their large size may be due to hydremia for it is well known that in hydremia the plasma is quite constant in its water-content and that the variations in the water of the total blood affect especially the cells. In pernicious anemia the largest cells are some times the darkest and some of the microcytes are exceedingly pale, while in secondary anemia the reverse is true (see plate I).

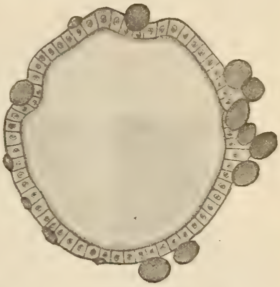

FIG. 135.—The intestine of an infected mosquito with oöcysts attached. (From Braun.)

STAINING PROPERTIES.—Red blood-cells like all other cells while "alive" are *achromatophilic*. If fixed by an agent which prevents post-mortem changes all that are normal are monochromatophilic and, since they take from a mixture only acid stains, are *acidophilic*. Since it is the hemoglobin that takes the stain, the amount of this pigment may be estimated from the depth of their color.

Red cells which either as a whole or in part take other than the acid component of a stain are termed *polychromatophilic* or *basophilic*. Under this term we do not now include the basophilic granules to be described later. Eosin stains basophilic red cells more faintly than normal and if followed by a basic stain, such as hematoxylin, will be supplanted by it. Basophilic corpuscles are usually larger than the normal, have less biconcavity and often are poikilocytes. Stained with hematoxylin and eosin such cells take a violet tint; with Ehrlich's stain a fainter orange than normal, or a grayish color; with polychrome methylene blue they stain a bluish violet color.

Ehrlich explains basophilia as a coagulative necrosis, an " anemic degeneration." In favor of this view are, that other signs of degeneration also are present; that it can be produced in animals by inanition; that these cells appear within 24 hours after a hemorrhage, that is, before nucleated cells or other signs of regeneration have appeared; and that basophilia affect especially the megaloblasts and other red cells which are abnormal in size or shape. Another view is that the basophilic cells are young since young cells certainly are basophilic. They are met with in pernicious anemia, in the grave secondary anemias, especially those due to cancer, in the eruptive fevers, malaria, the purpuras and after various blood poisons.

Other cells are "fuchsinophilic" (Plate I, 35); that is, if stained with Ehrlich's triple stain they are too red. Since so many of the nucleated reds of the bone marrow are fuchsinophilic this also is considered a sign of a young cell. These cells also are usually distorted, as if very soft. The same is true of the basophilic cells and nearly all nucleated red cells are slightly basophilic. Basophilia and fuchsinophilia however are not the same. With the blood stains now in common use we can disregard fuchsinophilia but are even more confused by basophilia since methylene blue, the basic stain used, is exceedingly untrustworthy.

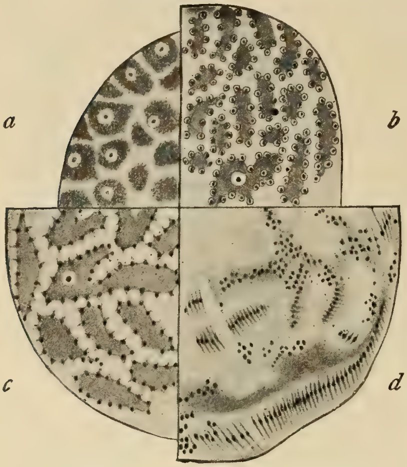

FIG. 136.—Various stages of the development of Plasmodium precox in the mosquito's stomach. *a*, In four to four and a half days after the bite; *b*, *c*, five to six days; *d*, eight days (Plasmodium vivax). (From Braun.)

Using the best of stains we may say that young red cells in general are basophilic and that many degenerating cells become so. For this reason it is hard to say whether microcytes, macrocytes and the basophilic granules are signs of regeneration or degeneration, but it is improbable that they have always the same significance. Theobald Smith, in describing a case of purpura, first suggested that basophilia is evidence of the youth of cells and later emphasized this in his studies on Texas fever.[25] Walker (see p. 862) found basophilic cells in the normal blood of all lower vertebrates, while in the blood of the fetus of the dog and guinea-pig there were even 90 times as many such as in the blood of the mother. In normal human marrow the basophilia of a cell is in inverse proportion to the amount of hemoglobin which it seems to contain hence the term "anemic degeneration"; but this lack of hemoglobin could be primary as well as secondary.[26] Walker suggests that these are "cells hurried into the circulation while too young" and therefore are as good and certainly a much more convenient index of anemia than is the blood-count.

Partial polychromatophilia is best illustrated by the definitely basophilic areas of Maragliano's endoglobular "degeneration" (see p. 448). The probability is that many of the so-called "inner bodies," "nucleoids," and other so-called evidence of cell-structure are nothing but these areas of changed protoplasm. Sometimes the areas markedly resemble malarial parasites, while others when extruded resemble platelets. These degenerations certainly led to many mistakes in the diagnoses of malaria before the chromatin-staining mixtures were used.

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[25] Walker, *loc. cit.*

[26] See also Stengel, Contrib. from Pepper Lab., Univ. of Pa., 1900.

The ring bodies described by Cabot [27] in some of the red cells of anemic blood and which require for their demonstration the polychrome methylene blue-eosin mixtures, he suggests are nuclear remains. These appear as rings, ovals, or bands and apparently are not related to the basophilic stippling. They occur especially in pernicious anemia but also in the leukemias and in various secondary anemias.

In specimens heated too quickly many of the cells have at their periphery a row of large dots which are not true granules.

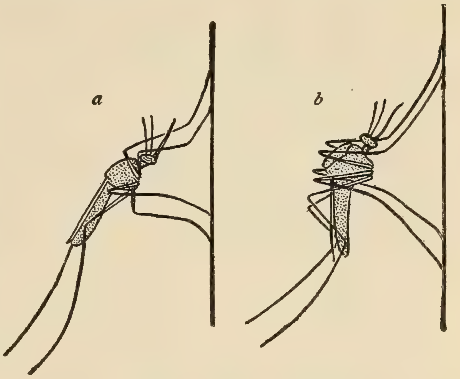

FIG. 137.—Attitude of mosquitoes on wall. *a*, Anopheles; *b*, Culex.

In certain cases of malaria (those we have seen all have been tertian and from the Tropics) the infected cells show a remarkable granulation (Plate III, 10, 13). These granules are of quite uniform size about 1μ in diameter. They can be seen in the fresh unstained cell (the lead granules cannot). They stain purple in the Hastings' stain, while the rest of the cell stains paler than normal, in fact may be almost colorless as if the hemoglobin had been condensed into these dots. We have seen red cells in which these granules appeared suspended in a hyaline envelope around the parasite.

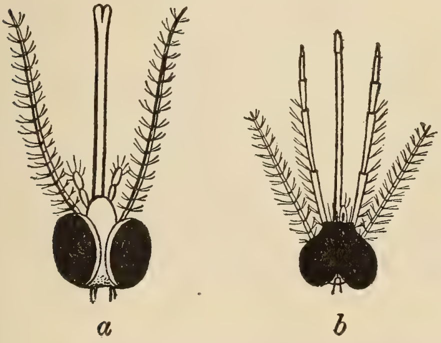

FIG. 138.—Heads of mosquitoes. *a*, Culex; *b*, Anopheles.

The "methylene blue degeneration of Ehrlich" is the name given to a beautiful blue mesh-like fibrillation of red cells in specimens of fresh blood stained by this dye.

Vital Blood Staining. — To study the granules and fibers of unfixed cells one drops on the smear before it dries a granule of methylene blue or neutral red and then immediately seals the cover glass to the slide with paraffin. Beautiful threads of fine granules are soon seen. Another excellent method of vital blood staining was that used by Rosin [28] who covered a cover-glass lightly with a saturated alcoholic solution of methylazur or of toluidin blue, which he then allowed to dry, and made the blood smear on this stained surface which was then at once inverted over a hollow slide with vaselined rim. The changes in blood thus prepared can be studied for even 24 hours.

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[27] Jour. of Med. Research, 1903, vol. lv., p. 15.

[28] Rosin and Bibergeil, Zeitschr. f. klin. Med., 1904, vol. liv, p. 107.

Vogel and McCurdy [29] recommended the following method:

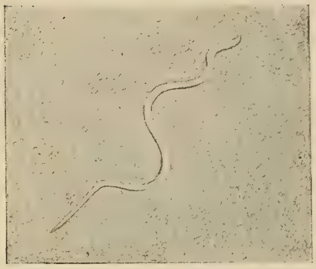

FIG. 140.—Filaria bancrofti. × 50.

One saturates 0.85% salt solution with brilliant cresyl blue (see below.) This is filtered through a double paper to take out the excess of dye substance and to prevent precipitation on the slide. It is better to centrifugalize the stain before using it in order to be sure that no undissolved particles remain in suspension. If any precipitate is present in the stain it later will be thrown down when centrifugalizing the mixture of blood and stain and will cause confusion when counting the reticulated cells.

The stain should be freshly prepared for use as follows: Saturated solution of brilliant cresyl blue in 0.85% salt solution and

Salt solution, 0.85% .... aa 5 c.c.
Sodium oxalate solution, 2% .... 2 c.c.

Add the oxalate to the salt solution, then mix with the stain and filter.

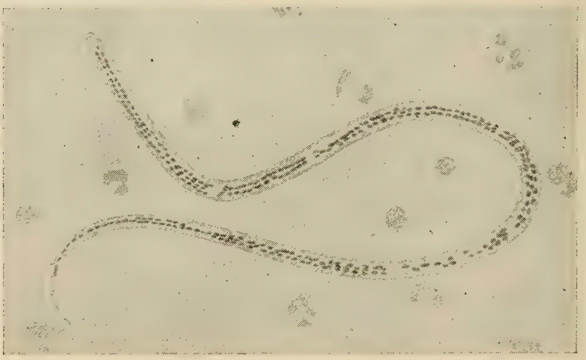

FIG. 141.—Microfilaria bancrofti with sheath, from blood taken at Charleston, S. C. Stained with hematoxylin. Travis, U. S. Public Health Service

The finger is so punctured that we can get a free flow of blood. A good sized drop is drawn into a red cell-counting pipette using the stain as a diluent. After thorough mixing, this is allowed to stand for 10 minutes and the contents of the mixing chamber then blown into a centrifuge tube and centrifugalized. The staining fluid is drawn off with a capillary pipette until only the sediment of cells remains, and these are then drawn from the bottom of the centrifuge tube in a capillary pipette. A drop of this fluid is placed on the end of a clean slide which has been slightly warmed in a flame and spread as in making ordinary blood-smears. Beautiful threads of fine granules are soon seen in certain cells. The preparation will keep indefinitely if mounted in neutral balsam or damar and not exposed to strong daylight.

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[29] Arch. of Int. Med., Dec., 1913, xii, p. 707.

In counting the cells an Ehrlich eyepiece is of great assistance. In suitably stained specimens the granulo-filamentous or reticulo-filamentous substance appears in the form of granular particles which are sometimes discrete but more often form threads which frequently are woven into skeins or wreaths of great complexity and which fill a considerable portion of the cells. In the blood of infants these reticulations are found in from 5 to 10% of the erythrocytes and in normal adult blood in from 0.5 to 2%. In severe anemias, however, their number may run as high as 18 or 20% and in hemolytic jaundice, where they are most numerous, they may occur in still greater proportions.

Vogel and McCurdy conclude that this granulo-filamentous substance is not derived from the nucleus, is different from polychromatophilia and from the basophilic stippling seen in fixed preparations stained with panchromatic dyes, is not a preformed structure but a precipitation product of the stain and is an evidence of youth of the cells and not of degeneration. In conditions in which a severe drain on the erythrocytes is being sustained by a well-functionating bone-marrow, large numbers of reticulated cells are found, whereas in aplastic cases they may be diminished almost to the point of absence. That is, the reticulated cells, in a manner somewhat comparable to the behavior of the erythroblasts, afford a direct insight into the hematopoietic activities of the bone-marrow. For clinical purposes they form a more convenient measure of this function than do the nucleated cells as their percentage relations to the erythrocytes can be more easily and accurately determined and their enumeration is to be urged as a part of the study of the blood in all cases of severe anemia.

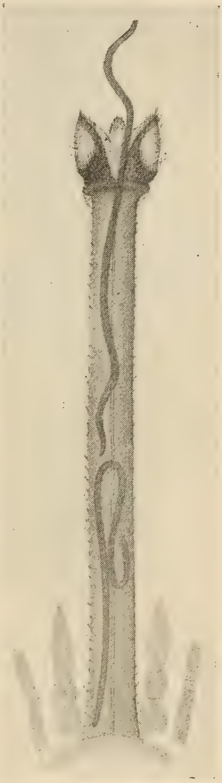

FIG. 142.— Mature larva escaping from proboscis of Culex fatigans. One mature larva coiled in base of proboscis. From mosquito dissected after being infected at Charleston, S. C. U. S. Public Health Service.

THE BASOPHILIC GRANULATION OF GRAWITZ (Plate II, 22, 24, 25).—In certain conditions, especially lead poisoning, pernicious anemia, leukemia, etc., certain of the red-blood cells when stained with any basic stain, but particularly with gentian violet or methylene blue, are seen to contain minute granules. These granules are not visible in fresh unstained specimens and do not increase in specimens on standing.

They are best demonstrated as follows: The air-dried smears are fixed for from 3 to 5 minutes in absolute alcohol, washed in water and, while still wet, are stained for a few seconds or much longer with Löffler's methylene blue. They are dried, or examined, while still wet. The bluish-black granules will stand out against the clear green corpuscles.

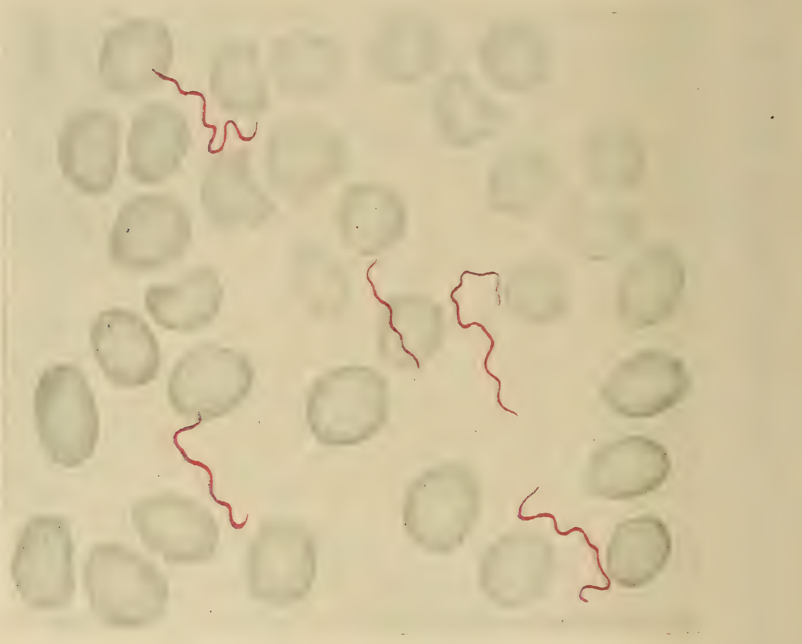

FIG. 143.—The spirochete of relapsing fever. × 1200.

Pappenheim proposed a stain intended to differentiate them from nuclear fragments. Stain I. Acid. carbol. liquefact., 0.25; aqua dest. 100; methylene green (pur.), 1. Stain II. Acid. carbol. liquefact., 0.25; aqua dest., 100; pyronin (pur.), 1.

Fifteen cubic centimeters of I and 35 c.c. of II are well mixed and filtered. The blood-smear fixed by heat (not alcohol) is stained for a few seconds with this filtrate. Nuclear fragments will stain a deep greenish-blue color, Grawitz's granules a bright red.

When numerous, 5 or 6 of these "stippled cells" may be seen in a field, but as a rule several fields must be searched in order to find 1 such cell. The cell may contain but 1 or a few of these granules, but as a rule it is well sprinkled even to such a degree that some are almost uniformly blue. The granules vary from dust-like size to 1μ or more in diameter. They may occupy any part of the cell, but are uniformly distributed as a rule and (many think) are situated in the external layers of the protoplasm. Stippled cells are met with in the severest anemias, especially the primary pernicious (in which they are large and conspicuous) and in secondary anemia especially that due to cancer of the gastrointestinal tract; in cachexia; in leukemia, in which cases they are not numerous; in septic processes; and in chlorosis (although some say they are rare, others as Stengel and Pepper who found them in 2 of 18 cases, say they are common); in phthisis, especially after the secondary infections develop; in lues; chronic parenchymatous nephritis; small contracted kidney; cirrhosis of the liver (Grawitz); in gout (in which condition they may be numerous, especially in cases with hematoporphyrinuria, and yet in other forms of arthritis with even severe blood changes they are very rare); typhoid fever; pneumonia; lues; nephritis; etc. Guyot found them regularly in a case of hemoglobinuria due to cold. They are found in the blood of Europeans who recently have moved to the Tropics.

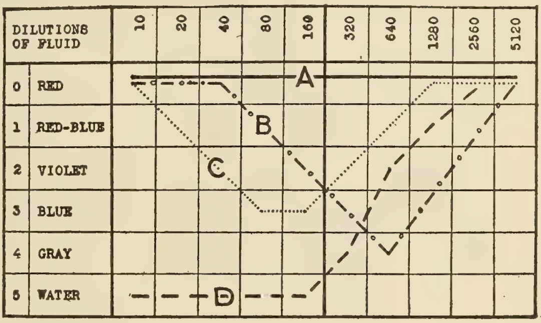

FIG. 144.—Colloidal gold curves.

But that condition in which they are particularly important and numerous is lead poisoning, since no other blood changes need be present. Some claim that they are present in the blood of all lead-workers, but they certainly cannot always be found in the time at the disposal of the ordinary clinical worker. They vary much in number from day to day. As a rule they appear early, even after but 4 days' exposure to lead, and they may persist in the blood for over 20 years. Since these granules may be the first sign of an anemia we may possibly diagnose this condition even before the count drops. They also are the last sign to disappear.

Grawitz interpreted these granules as areas of coagulated necrosis, hence the name "Grawitz basophilic granular degeneration," and Hamel,[30] White and Pepper,[31] Stengel and Pepper,[32] Bloch and others agree.

On the other hand similar granules are found in the blood of the embryo, and nucleated reds often contain them, which is good evidence that some at least are not nuclear fragments and that they bear some relation to blood regeneration.

Cadwalader [33] distinguished 3 groups of granulated red cells: those with the granules in fine and coarse thread-like strands, found sometimes in normal blood; those with fine dot-like granulations, the most common form, especially in lead poisoning and pernicious anemia; and those with dense coarse basophilic masses seen in cases of lead poisoning in which nucleated reds are plentiful and suggesting by their position and size fragments of a nucleus, although the transitional stages between fragmenting nuclei and these granules are not found and they are rare in the bone-marrow where karyorrhexis is most common.

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[30] Deutsch. Arch. f. klin. med., May 23, 1900.
[31] Am. Jour. Med. Sci., Sept., 1901.
[32] Am. Jour. Med. Sci., May, 1902.
[33] Bull. of the Ayer Clin. Lab., Univ. of Pa., January, 1905.

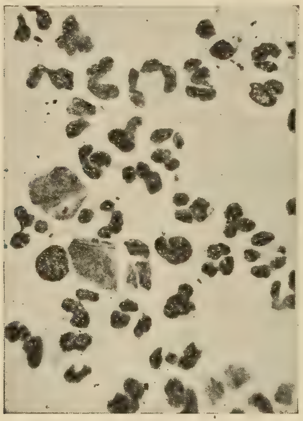

FIG. 145.—Smear of the spinal fluid of a case of epidemic cerebrospinal meningitis.

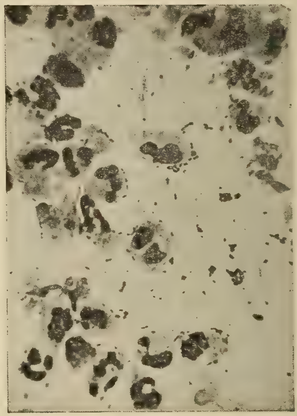

FIG. 146.—Smear of the spinal fluid of a case of meningitis due to Diplococcus lanceolatus.

At this point we take the liberty to state that practically every basophile granule found in red blood-cells has been described by one or another writer under this one title. In our opinion there are at least 3 different basophilic granulations in red blood-cells, which have, we suspect, no relationship the one to the others.

The granules seen in lead poisoning are not visible in fresh blood; they are seen also in normoblasts with perfect nuclei. These are always accompanied by other evidences of blood regeneration. The granules seen in malaria are described on page 668. They can be seen in the fresh cell. Compared side by side with the Grawitz granules they do not look at all alike. The granules described by Vaughan (see page 449) as remnants of nuclei do not resemble the Grawitz granules. We judge from Cadwalader's figures that he includes them as his coarse variety.

Naegeli [34] considered them related to blood regeneration and Boellke[35] denied that they bear any relation to the nucleus.

Number of Red Blood-cells.—The average count of the red blood-cells of the normal adult man is usually given as 5,000,000 per cubic millimeter of blood; of the woman, 4,500,000. In a healthy young man, however, it is more common for the count to vary from 5,000,000 to 6,000,000.

By *polycythemia* is meant a condition with more cells than this per cubic millimeter; by *oligocythemia*, one with a smaller number. It is evident that such numbers are simply relative, that variations in the count may be due either to actual variations in the total number of red blood-cells in the body or to variations in the amount of plasma.

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[34] Münch. med. Wochenschr., 1904, No. 5.

[35] Virch. Arch., 1904, vol. clxxvi, S. 47.

The blood-count may vary in different parts of the body. Oliver[36] found that anything which increases the blood-pressure locally causes a rise in the count at that point. For instance, the count is higher in a limb that has been hanging in a dependent position and in areas subjected to active or passive motion.

Exercise of a part will raise the count locally and the application of cold and of heat will lower it or raise it, according as it produces stasis, vasodilatation, or constriction.

Excessive exercise, Willebrand found, would raise the general count of red cells from 3 to 23% (average 12.3%), and that of leucocytes from 19 to 97% (average 47%).

Physiological Variations.—The *sex variations* have already been mentioned. This difference is present only during the menstrual period of life, since the count of girls until their fifteenth year averages 5,444,000 and for boys of the same age 5,102,000; also between the ages of 40 and 60 the count of women averages 5,000,000.

The red cell count varies much with *age*. The maximum is at birth, in which case it may be even 7,000,000 but as a rule is lower—*e.g.*, 5,740,000 (Stengel and White). Otto found the average for the first 4 days to be 6,155,000; in 1 child 10 hours old it was 6,910,000. After the first 4 days of birth the count begins to drop and reaches a minimum in about 1 year (see below). This count depends somewhat on the time at which the umbilical cord is tied, since tying it off late may result in a gain of almost 1,000,000 cells per cubic millimeter. These high counts at birth are due probably to an unusual concentration of the blood resulting from the loss of water. They last but for a few days, not over 10, after which time the nucleated reds also disappear.

From birth until about the tenth year the count is relatively low and then slowly rises. There is considerable difference of opinion as to the exact age when it reaches its minimum. It rises from the time of puberty to 30 years of age, during which period the count in young healthy persons often ranges from 5,500,000 to 6,000,000 cells. Between 30 and 50 years a count of 5,000,000 for men and 4,500,000 for women may be considered normal. After 40 the count is inclined to drop slowly in men and to rise in women.

Not satisfied with the age curves usually quoted in text-books, we have tabulated the counts of all patients with apparently normal blood and those of our medical students (see page 444).

We have calculated means, not averages, since the extremes are always subject to criticism and should be reported independently. For a discussion of these low hemoglobin estimations see page 444.

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[36] Brit. Med. Jour., 1896.

BLOOD OF PATIENTS
MALES

Years	Cases	Reds (mean)	Hb mean Per cent.	Index	Leucocytes
6 to 15	5	5,560,000	85	....	7500
16 to 25	36	5,200,000	85	0.8	6500
26 to 35	69	5,300,000	90	0.85	7000
36 to 45	42	5,500,000	90	0.82	5500
46 to 55	21	5,300,000	80	0.75	9000
56 to 65	9	5,000,000	80	0.8	....
66 and over	5	4,000,000	60	0.77	7500
		FEMALES			
10 to 15	5	5,000,000	75	0.75	8000
16 to 25	43	4,500,000	77	0.85	7500
26 to 35	55	4,500,000	80	0.88	7200
36 to 45	34	4,600,000	72	0.80	7700
46 to 55	17	4,500,000	77	0.85	7000
56 to 65	10	4,500,000	70	0.78	6000
66 and over	3	4,700,000	65	0.7	7000

A study of the blood of 176 men students from 20 to 25 years of age gave the following: Means of reds, 5,000,000 (extremes 4,500,000 and 6,700,000); 14 (8%) were below 5,000,000 and 15 (8.5%) above 6,000,000. Mean of leucocytes, 7500 (52 cases); of hemoglobin, 14,5 gms. (Miescher), 92% (Fleischl), 95% (Dare), 92% (Gowers).

Women medical students, same age limits, 16 cases. Mean of red 4,800,000; of leucocytes, 8000; of hemoglobin, 11 gms. (Miescher), 85% (Fleischl), 87% (Dare), 82% (Gowers).

Nutritional Conditions.—The red cell count of thin muscular persons is somewhat higher than that of fleshy persons. A large meal may be followed by a temporary slight decrease, said to be due to an increase in the plasma. During hunger periods the count often rises a half million cells in 24 hours. This is attributed to concentration of the blood.

The *temperature* has an influence on the count. In winter the counts averaged about 500,000 cells per cubic millimeter more than in summer (this was well seen in some of our students' counts). The change of residence from temperate zones to the Tropics may lead to a drop in the count of from 500,000 to 2,000,000 cells.

Pregnancy.—The counts of pregnant women and also of the fetuses are said to drop during the last part of pregnancy; that of the mother about half a million cells and the hemoglobin 20%. The count of the fetuses of from 7½ to 8½ months was found to be 7,000,000 and at 9 months 6,500,000 (Biondi and Gardini). The bloods of mother and child are independent enough so that if the mother has an anemia-producing disease the child can preserve its count fairly well, and *vice-versa.*

Thompson made a very careful study of the bloods of 12 pregnant women in Dr. J. Whitridge William's clinic. He found that the counts and the hemoglobin decreased slightly from the fourth to the eighth month and then rose to normal at term. The curve of the specific gravity of the blood ran in general parallel to that of the red cells and hemoglobin, but the initial fall and the terminal rise were more accentuated than of those curves. The minimum figures (1.0408) were during the sixth month.

Altitude.—That the red cell count rises as persons ascend to high altitudes is a phenomenon long ago witnessed but not yet thoroughly explained. The rise amounts to about 50,000 cells per 1,000 feet. The count returns to normal as they descend, or at the latest 36 hours after. The rise in the count is especially marked if the ascent to a considerable height is sudden. It is slight following an ascent of 1200 meters, slight and tardy after an ascent of 1800 meters, but it is immediate and considerable after an ascent of 3000 meters. This increase in the count is certainly too rapid to be explained by new blood formation and after the descent there are no signs of blood destruction. The rise is most marked in invalids, especially those with lung tuberculosis. The symptoms of anemia are even aggravated by the ascent.

There would seem to be 2 factors which raise the blood count in these cases, a temporary change in the distribution of the blood-cells and later, in 8 or 10 days, an increased production of new cells.

Miescher and his pupils, as a result of work with animals, claimed to demonstrate that a diminished oxygen tension is a stimulus to new blood formation. Later, practically all of this experimental work was challenged. Certainly animals sent to high altitudes have given disappointing results.

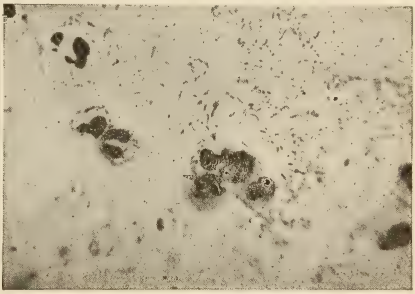

FIG. 147.—Smear of the spinal fluid of a case of meningitis due to Bacillus influenzæ.

Others (Grawitz) say that the blood is concentrated because of evaporation of body water, and yet the solids of the plasma do not change as much as does the count; others (Truntz) that it is due to an accumulation of cells in the capillaries; that the cells lived longer; that there is at first a fragmentation of the red cells which explains the early increase and then a true new formation follows (Koppe); finally that there is a peripheral vasoconstriction which causes an increase in the tissue lymph and so a concentration of the blood (Bunge). One of the best papers on this subject is that of Campbell and Hoagland [37] who consider that the rise in the count is explained largely as a change in the distribution of the blood cells which results from the lowering of the blood pressure and is due to the lowered barometric pressure and to a compensatory increase in the heart action, the increase in pulse rate being almost parallel to that of the count. Mosso showed that there is at high altitudes a peripheral vasodilatation, hence a stasis in the dilated capillaries. If a person remains at the high altitude the heart will soon compensate for all these factors and the count return to normal. The difference in temperature has some, some say the most, influence on the blood count, hence at Colorado Springs it is about

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[37] Am. Jour. Med. Sci., November, 1901.

800,000 cells per cubic millimeter lower than at the City of Mexico, although the 2 places have the same elevation. Experiments with rabbits showed a decreased count in the mesenteric circulation corresponding to the rise in that of the peripheral circulation.

The observations of Gaule, who studied his blood during a balloon ascension and found that the rise was accompanied by the appearance of many nucleated reds, have not been confirmed.

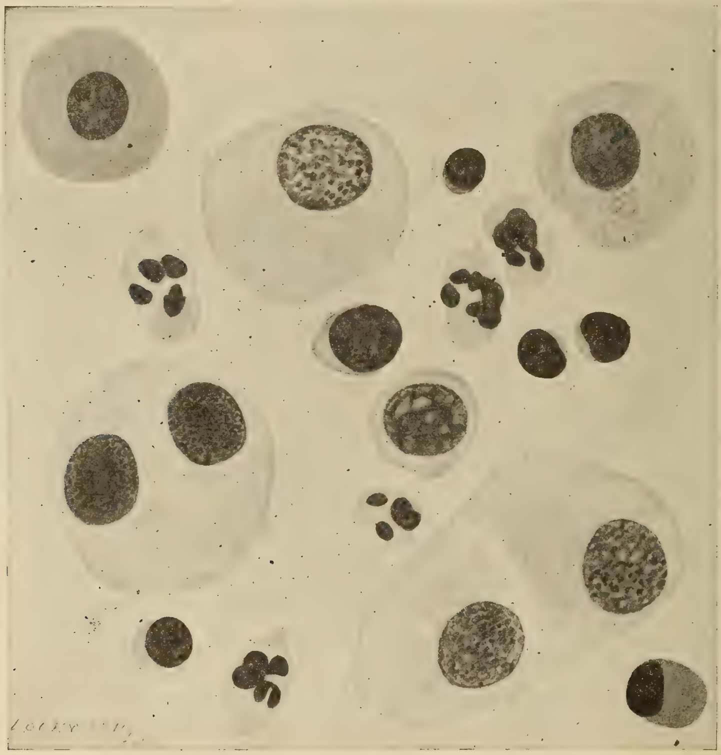

FIG. 148.—Cells from a pleural exudate. × 900.

Drugs and Therapeutic Measures.—Among the drugs which increase the count of the red cells are iron, which is almost a specific remedy in chlorosis, and arsenic, a drug equally valuable in pernicious anemia. Mercury in large doses causes an anemia. The destruction of injured cells by this drug may explain the Justus test for lues (see page 654). Lead causes a chlorotic anemia which some attribute to a direct injury of the red blood-cells (see page 481).

Any drugs which can change the amount of plasma by causing rapid losses of fluid to the body, as diuretics, emetics, purgatives, diaphoretics, may cause a temporary rise in the count; but this is hard to demonstrate in the wards.

Cold baths in typhoid fever cause an average increase of 1,860,000 in the red cell-count and an increase of the specific gravity of the blood (Thayer) which disappears in about 1 hour. This is due to a temporary stasis in the capillaries. Breitenstein thinks the effect of a cold bath is greater in a typhoid patient than in a normal man since the distribution of cells in the former is abnormal before the bath.

After operations there is often a transitory rise of from 100,000 to 1,000,000 cells, probably due to changes in the peripheral circulation.

Pathological Conditions.—The toxins of certain of the specific fevers often cause a marked anemia. This may be due to a definite destruction of red cells, in which cases there is an increase of the pigment in the urine, and sometimes, although rarely, to definite minute or larger hemorrhages. In other cases changes in the bone-marrow may be important.

Chronic Cachexia.—The toxins of the more chronic infections which produce chronic cachexia may produce a definite anemia. Of these, tuberculosis, cancer and lues are the best illustrations (see pages 634, 648, and 652). The methemoglobin-producing poisons may diminish the count because of their direct destruction of the cells. Among these are pyrogallic acid, the chlorates and certain of the coal-tar products, as antifebrin and phenacetin.

Polycythemia.—In this section we follow closely the excellent article by Watson-Wemyss.[38] Polycythemia, an increase in the number of red corpuscles per cubic millimeter of blood, may be transient or permanent. The transitory polycythemia is usually due to a concentration of the blood caused by loss of fluid from the plasma; to local cyanosis, to poisoning with acute phosphorus (in which case the count may be even 8,650,000) and carbon monoxide (the count even 6,630,000). Vomiting alone cannot explain these high counts.

Permanent or absolute polycythemia would seem to be the result of an absolute increase in the production of red cells. In favor of this view are the signs of active blood formation (nucleated red cells, polychromatophilia, leucocytosis, eosinophilia, etc.). This polycythemia may be a secondary process (erythrocytosis) or a primary process (erythremia).

Erythrocytosis, or a permanent polycythemia, the cause of which is in part at least understood, may be due to high altitudes; heart diseases, especially the congenital forms in which the count may vary from 8,000,000 to 9,000,000 per c.mms.; also mitral-valve disease and adherent pericardium, lung diseases, especially empyema, acute miliary tuberculosis and pneumonia; chronic stimulation of the bone-marrow by poisons, as phosphorus, acetanilide, etc.

Erythremia is considered a primary disorder since we cannot ascribe it to any known cause. It seems due to abnormally increased activity of the marrow and is often accompanied by enlargement of the spleen, cyanosis and sometimes by arterial hypertension. This condition has been named Vaquez's disease, Osler's [39] disease, splenomegalic polycythemia, etc. The red cell-count of these cases varies from 6,000,000 to 13,000,000. The red cells are of normal size, nucleated red cells are not rare and poikilocytes and polychromatophilic cells are sometimes seen. A polymorphonuclear neutrophile leucocytosis (from 20,000 to 91,000) is the rule although a leucopenia has been found. There is also an absolute eosinophilia. Myelocytes are rarely found.

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[38] Edinb. M. J., Feb., 1911, vol. lv, N. S., p. 129.

Osler reviewed 9 cases, 4 of which he reported. The cyanosis was extreme and lasted even for years; the highest count was Koester's of 13,600,000 and but 1 was below 9,000,000 (8,250,000); the hemoglobin ranged from 120 to 150%, the specific gravity from 1.067 to 1.080 and the leucocytes from 4000 to 20,000 (the most of the counts were below 10,000). Zamfirescu reported this condition in a woman with polycythemia, cyanosis, dyspnea and cough. Kikuchi one with bronchiectasis. Türk [40] reported 7 cases like Osler's, 2 with autopsy, the counts of which varied from 7,700,000 to 10,600,000. He suggested that erythremia is due to a primary hyperplasia with increased function of the erythroblastic myelogenous tissue and so is analogous to leukemia. Other interesting cases of polycythemia without cyanosis have been reported, as Zandy's [41] who proposes the term "erythrocytosis." Türk [42] mentioned cases characterized merely by the high count.

In this connection we should remember that we usually count capillary and not arterial or venous blood and that the count in the capillaries may not be the same as that in the vessels although it usually is the same as that in the veins. To appreciate this important point the student should study the capillary field of a frog's mesentery or a rabbit's ear. He will see some wide capillaries, some through which the corpuscles pass in single file and others so narrow that the cells do not enter them at all. If now an active congestion or a venous stasis be produced the capillaries fill up with cells, even those which before transmitted only plasma. In this local area a count of capillary blood certainly would be higher than before.

Local changes in counts are much more marked in the case of the leucocytes than of the reds since the former collect in the smaller vessels, forming layers along the walls.

Local cyanosis may deceive one much; for example, some cases during life have normal blood-counts who at autopsy show a condition suggesting pernicious anemia. The same is true of certain dysenteries.

High counts are met with following the use of various coal-tar products.

One of our students recently became cyanotic after handling aniline oil. His red cells then were 5,900,000, hemoglobin 107% (Dare) and leucocytes 6100. Six days later the red count was 5,084,000 and the hemoglobin 78% (Dare).

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[39] Am. Jour. Med. Sci., 1903, vol. cxxxvi.

[40] Wien. klin. Wochenschr., 1904, Nos. 6 and 7.

[41] Münc. med. Wochenschr., 1904, No. 27.

[42] Deutsch. med. Wochenschr., 1904, No. 20.

Resistance of the Red Blood-cells.—Many methods have been proposed for determining the resistance of the red blood-cells in the hope of explaining such phenomena as hemoglobinemia, etc. At first the methods used were mechanical, such as shaking, chemical and electrical, but now they are biological.

Hamburger's Method.—Formerly the resistance of the red cells was tested by exposing the washed red cells to salt solutions of various strengths to determine the concentration at which hemolysis takes place. In 16 small test-tubes are mixed 1 drop of a suspension of the washed corpuscles and 1 c.c. of a series of sodium chloride solutions, the lowest of which is an 0.4 % solution and each succeeding one 0.03% higher than the preceding one. The tubes, after a gentle shaking, are allowed to stand 6 hours. Normal blood plasma is isotonic with an 0.9% NaCl solution, but normal corpuscles are not laked unless the concentration is less than about 0.4%. In congenital family cholemia the hemolysis may begin at 0.7 or even at 0.9%. Stengel diluted the blood 1, in a Zeiss leucocyte pipette, to 10, with sodium chloride solutions varying from 0.42 to 0.52% shaking the mixtures well. The blood is then blown into small tubes sealed at one end. These after

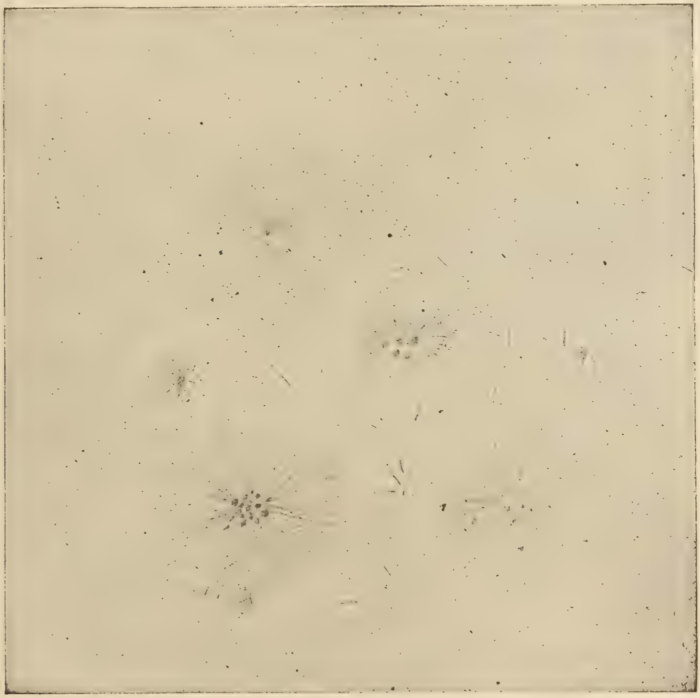

FIG. 149.—Fatty acid crystals from the contents of an ovarian cyst. × 400.

standing are centrifugalized for from 2 to 5 minutes, then held against a white paper to determine the presence of hemolysis. Stengel found that saturation with an excess of carbon dioxide (as in congestion) causes no morphological alteration in the cells and yet increases their vulnerability; that cold produces marked changes; and that hypotonic salt solutions have the power in the test-tube as well as within the blood vessels of decolorizing and vacuolating the red blood-corpuscles. (This point is disputed by many workers, who find that even the intravenous injection of distilled water does not lake any blood-cells). Heat of even slight degree changes the shape and size of the corpuscles and finally decolorizes them. A higher degree causes budding, vacuolation and, a somewhat higher degree, complete fragmentation (see page 446).

MECHANICAL INFLUENCES.—In some conditions the cells have been found to have decreased resistance to shaking. Meltzer has shown [43] that the effect of shaking depends upon the rapidity of vibration, that for each blood there is a minimum and maximum rate which the cells can bear without destruction. Laker tested the cells by passing through their suspension the discharges of a Leyden jar. Various other methods have been proposed, but with as yet little result. For the much more important BIOLOGICAL METHODS see page 588.

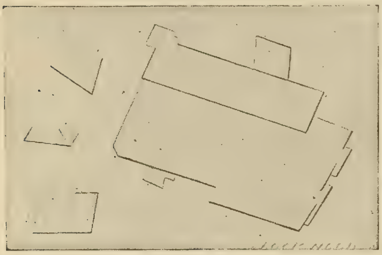

FIG. 150.—Cholesterol crystals. × 400.

Color Index.—By color index is meant the percentage of hemoglobin divided by the percentage of the red blood-cells. This figure, as Duncan first showed, has considerable value. For the denominator, 5,000,000 red blood-corpuscles is considered 100%, while the numerator is the per cent. of hemoglobin read with any good instrument. The color index is less than 1 in practically all secondary anemias. It is especially low in chlorosis, averaging about 0.5, and in some cases reaching as low as 0.3. In pernicious anemia, on the other hand, the index is increased, averaging about 1.04 (Cabot) and in 1 case reaching 1.75 (count 1,000,000, hemoglobin 35). The high color index is of value in differentiating pernicious anemia from certain cases of latent cancer of the stomach, a diagnosis often hard to make. The color index is not a strict mathematical calculation: the 2 figures are too approximate for that. Five million is only approximately the normal red-cell count and few hemoglobinmeters read normal blood at 100%. As stated on page 484 we found, taking instruments as they come, that the index in normal persons varies from 0.80 to 0.88. The color index in the case of our male students, using the Fleischl and Gowers instruments, was 0.84 and with Dare, 0.87. The index of the blood of our women medical students using the Fleischl was 0.88, the Dare, 0.9 and the Gowers, 0.82. Yet we often see persons with the above figures reported as cases of "mild chlorotic anemia."

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[43] Johns Hopkins Hosp. Rep., vol. ix.

We therefore approached the question in still another way. Among our students' reports were 53 records of counts and Miescher hemoglobin estimations made on the same normal bloods at the same hour. The counts varied from 4,600,000 to 6,700,000, and the hemoglobin from 10.9 to 17.2 gms. per 100 c.c. If in each case the number of grams of hemoglobin per 1,000,000 cells be reckoned, the mean of these figures should be an approximately normal color index. These quotients fell within surprisingly close limits since 42 of the 53 varied from 2.2 to 2.8 gms., while the mean was 2.63 gms. per 1,000,000 cells. We believe this standard of hemoglobin content to be more accurate than the color index.

The more carefully blood counts are made, the better the instruments used for hemoglobin estimation, the more evident are the individual, daily, seasonal and racial variations. The regulation of the composition of the blood is wonderfully efficient. Although enormous amounts of water may pass through the vessels as in diabetes insipidus, of water and solids as in diabetes mellitus, of albumin and water as in cases of rapidly collecting ascites repeatedly tapped, yet the blood's composition in any individual case varies within comparatively narrow limits.

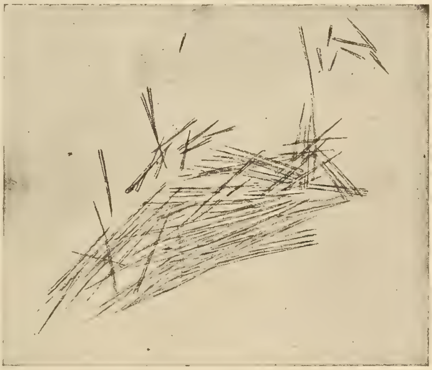
FIG. 151.—Sodium biurate crystals from a tophus. × 400.

The VOLUME INDEX or the quotient of the volume per cent. as determined with the hematocrit (considering a column of corpuscles of 50% as normal, see page 460) and the count per cent. (5,000,000 = 100%) promises to be of value.[44] The most important result of Capp's work in this field is that in pernicious anemia the color index never exceeds the volume index; that is, that there is no "supersaturation" of the corpuscles with hemoglobin but the high color index is due to an increase of the size of the cells alone. On the other hand, in secondary anemia the color index may fall below the volume index, while during regeneration the volume index may return to normal first. He has never seen any evidence of "acute dropsy" of the red cells.

WHITE BLOOD-CELLS

GRANULATIONS OF LEUCOCYTES.—By granules of leucocytes are meant in this section the minute bodies, usually spherical, with a size and staining character fairly constant for each granulation, which can be easily seen in the fresh blood and which seem to be inclusions of the protoplasm of the cell. All protoplasm is slightly and indefinitely granular but leucocyte granules are definite inclusions of the cell protoplasm which are liberated as independent bodies when the cell breaks up. They would seem to be a specific product of the secretory activity of the cell, not the result of a degeneration of the protoplasm or an accumulation of the products of metabolism, or inclusions from phagocytosis.

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[44] Capps, Jour. of Med. Research, 1903, vol. v.

Ehrlich classified the granulations as: first the *eosinophilic*, *acidophilic*, or *oxyphilic* granules (α). These granules are coarse, about 1μ in diameter, spherical or slightly oval, quite uniform in size and color and so refractive that in the fresh specimen they appear black. From a mixture of stains these always take the acid ingredient. These granules have been found in the blood of every animal from the frog to man whose blood has been examined. They are of albuminous nature and contain iron.

Amphophilic (β).—These granules are described as varying in size from that of α granules to others much smaller. Some are said to take acid and others basic stains. In a mixture of eosin and indulin, however, all the β granules will take the latter. They are sometimes met with in the same cells as α granules, hence Ehrlich considers them a younger stage of these. This is said to be the only case in which 2 specific granulations are found in the same cell. They are met with in some white cells of the bone-marrow of man and of various animals (rabbit and guinea-pig) and in the peripheral blood of patients with certain anemias. They may, in leukemia for instance, explain the variations in the size and tint of the granules of some of the eosinophile cells.

Basophilic and Mastzell Granules (γ).—In the connective tissue and blood of all animals and of man are cells which contain large basophile granules. Those found in the tissues are the true Mastzellen. These stain best with dahlia, taking a metachromatic rather than a strictly basophilic tone resembling the tint of mucin, of which, indeed, some claim that they are composed. This tint is best seen if polychrome methylene blue is used. These granules are spherical or oval in shape and vary considerably in size in the same cell. Cells containing somewhat similar granules are found in normal blood. These are increased in leukemia while in some cases of pleural exudate and of gonorrhea these may be the predominating leucocytes of the pus. Even Ehrlich admitted that these leucocytes may not be related to the Mastzellen of the tissues. They seem to differ in origin while their granules do not stain exactly alike. What is more, the γ granules in the cells of abnormal bloods are not exactly like those present in the cells of normal blood and of bone-marrow. Those in leukemia blood, *e.g.*, are more soluble in aqueous solutions than are those of normal blood. It is, therefore, at least possible that we have to do with 3 or more different granulations, or with the same granulations at different stages of their development.

Basophile Granulations (δ).—These granules were originally described by Ehrlich as occurring in the mononuclear cells, as not staining by dahlia, therefore not γ granules, and as occurring especially in the cells of bone-marrow. Later he seemed to consider them not as true granules but as nodes of the reticulum of the protoplasm.

Neutrophile Granules (ϵ).—These granules which are extremely fine and dust-like are seen in the mononuclear cells of the bone-marrow, a few in the mononuclear cells with indented nuclei of the blood stream, the endothelial leucocytes of later writers, but they fill the common polymorphonuclear cell of the blood. Stained with Ehrlich's triple stain, devised as a specific stain for them, they take a lilac color, but they also take an acid stain and so are called the " fine oxyphilic granulation," in contradistinction to the eosinophilic or " coarsely oxyphilic " cells. While a somewhat similar granulation occurs in the blood of cattle, swine and sheep (Hirschfeld) granules of exactly this size, arrangement and color are found only in man and hence they are considered by Ehrlich to be specific for man.

In addition to the above mentioned granules are others seen only in specimens stained with polychrome methylene blue mixtures in the mononuclear cells especially. The fact that these granules can be demonstrated is said by some to destroy Ehrlich's sharp distinction between granular and nongranular cells (see below).

Neusser's Perinuclear Granulation.—In certain specimens stained with Ehrlich triple stain are seen, in the mononuclear leucocytes especially, but sometimes in all forms of the leucocytes, blackish-green granules which always appear attached to the nucleus. They vary much in size. They have often a glistening or refractile appearance. Neusser considered them as characteristic of "the uric acid diathesis" but it has been shown[45] that they are in reality artefacts which can be produced by variations in the time of heating and of the stain, which with some mixtures may be produced at will and which bear no relation to the output of alloxuric bodies in the urine.

The Granulation of Lymphocytes.—In well-spread specimens stained by the various modifications of the Romanowski stain (but not by Ehrlich's stain) fine violet granules are seen in about ⅓ of the lymphocytes, especially those with a fairly wide protoplasm margin and also in some of the large mononuclear cells. They are not always spherical; their size is between that of the α and ϵ; few or many may be present in one cell, yet as a rule, they are not too numerous to count. They are not found in cells in smears of lymph-glands or of marrow. By their discovery Michaelis and Wolff[46] considered that they had broken down the sharp line of demarcation drawn by Ehrlich between the granular and the nongranular cells. Ehrlich replied that while it cannot be denied that these were granules yet they cannot be considered as forming a definite granulation in the sense in which he

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[45] Futcher, Centralbl. f. innere Med., 1899.
[46] Virch. Arch., Bd. 167, p. 151.

PLATE I

Cells of Normal Blood.

1. Small lymphocyte; small mononuclear.
2. Eosinophile leucocyte.
3, 4. Large lymphocytes.
5. Transitional mononuclear.
6, 7. Polymorphonuclear neutrophile leucocytes.
8. Mastzell.

Cells Found in Splenomyelogenous Leukæmia.

9, 11, 17. Neutrophile myelocytes.
10. Dwarf polymorphonuclear neutrophile leucocyte.
12, 13. Transitional cells between myelocytes and polymorphonuclear cells.
14. Eosinophile leucocyte.
15. Lymphocyte.
16, 19, 20, 21. Large mononuclears.
18. Dwarf polymorphonuclear eosinophile leucocyte.
22. Polymorphonuclear eosinophile leucocyte.

Erythrocytes in Chlorosis.

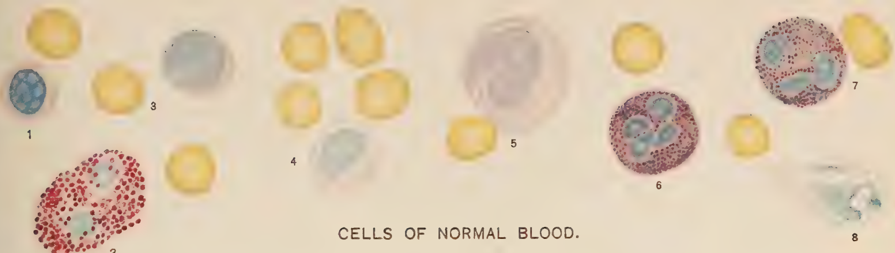
23. Cells found at the height of the disease. These are the "doughnut" or "pessary" forms.
24. Cells from the same case as 23 during convalescence.

Poikilocytes in Pernicious Anæmia.

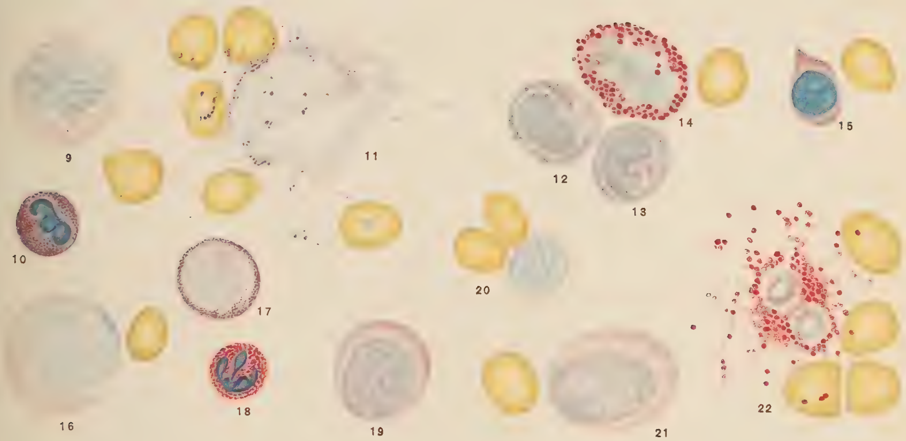
25. Battle-door form.
26. Sausage form.
27. Microcyte.
28. Megalocyte.

Nucleated Red Blood Cells.

29. Mature normoblast.
30. Immature normoblast.
31, 32. Intermediate forms.
33. Megaloblast.
34. Normoblast with nucleus showing fragmentation or incomplete mitosis.
35. Fuchsinophilic normoblast.
36. Leucocyte of the same size as 37 shown for comparison.
37. Large nucleated red cell.
38. Intermediate nucleated red (or megaloblast), the "reptilian form."



CELLS OF NORMAL BLOOD.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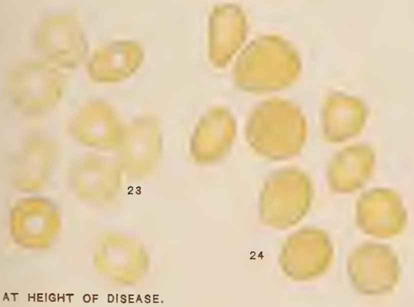
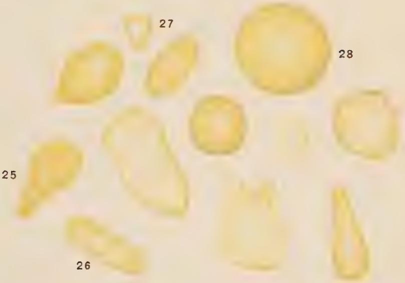
SPLENO-MYELOGENOUS LEUKÆMIA.



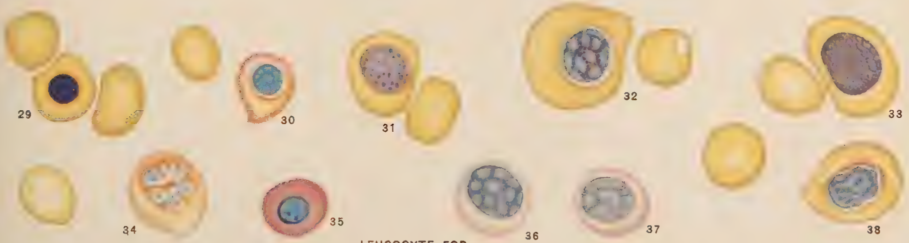
AT HEIGHT OF DISEASE.

ERYTHROCYTES
IN CHLOROSIS.

SAME CASE DURING
CONVALESCENCE.



POIKILOCYTES
IN PERNICIOUS ANÆMIA.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ALL STAINED WITH EHRLICH'S TRIPLE STAIN
AND DRAWN TO SAME SCALE.

LEUCOCYTE FOR
COMPARISON.

NUCLEATED RED BLOOD CELLS.

F. S. Lockwood.

LARGE MONONUCLEARS.—The cells of this group may vary in size from those as small as lymphocytes to others the largest cells in the blood. They have a large, oval, vesicular, eccentrically placed, faintly staining nucleus, which indeed may be overlooked, and abundant weakly basophilic protoplasm with or without a few granules according to the stain used. The larger cells of this group make up about 1% of the leucocytes of the normal adult blood (Ehrlich) but the entire large mononuclear group varies from 5.6 to 8.1% average 7.2% (Miller 10.8%.) In normal blood the large cells form a definite group of almost uniform size but in pathological conditions, especially leukemia, typhoid fever and malaria, this group may be represented by cells of all sizes from that of lymphocytes to large giant-cells. (Plate II. The group 9–20 contains many.)

Those of this group with much notched nucleus, the so-called " wallet " or " saddle-bag " nucleus, were called by Ehrlich *Transitional cells* (Plate I, 5), since he at first considered them intermediate stages between large mononuclear and polymorphonuclear finely granular cells. Although he soon abandoned this opinion (see page 450) the name is still in use. These cells are the largest of all in the normal blood. The protoplasm stains quite deeply and often contains a few neutrophile granules. These cells average from 1 to 3% of the leucocytes of normal blood. They are but older forms of the large mononuclear group.

Phagocytes of the Peripheral Blood.—Evans called attention to the occasional presence in the blood of large mononuclear leucocytes derived directly from the endothelial cells lining the walls of the capillaries in spleen, lymph glands and bone-marrow, which are definitely phagocytic in character. These he called endothelial phagocytes. He denied their presence in normal blood. Later McJunkins claimed that these not only were present in normal blood but that even 7% of the leucocytes normally are these.

McJunkin's Method for the Demonstration of a Phagocytic Mononuclear Cell in the Peripheral Blood.[48]—Three cubic centimeters of blood are added to 2 c.c. of a 3.8% sterile sodium citrate solution that contains 1% by weight of a good commercial grade of lampblack. The citrate-lampblack liquid is previously shaken vigorously in a flask to secure as even a suspension as possible and the 2 c.c. are measured at once into 15 c.c. graduated centrifuge tube. To this the blood is added as quickly as possible. To obtain the blood the palmar surface of a finger tip is painted with tincture of iodin, wiped with 95% alcohol, dried, punctured deeply with an automatic lance and the blood allowed to drip directly into the tube. This citrated blood is mixed thoroughly by striking the lower end of the centrifuge tube with the finger and is then immediately filtered through a single layer of freshly laundered muslin into a second centrifuge tube in order to remove the gross particles of lampblack. This second tube may be prepared in advance by autoclaving it with the cloth pressed into its upper portion in the form of a cone. The cloth is moistened with a 3.8% citrate solution before the citrated blood is poured onto it. It is not advisable to filter the suspension of lampblack before adding the blood because it tends to filter clear.

The filtered citrated blood is centrifugalized at a moderate speed for 15 minutes and at a high speed for 5 minutes. The tube is removed from the centrifuge and the black layer of leucocytes on the surface of the corpuscles is carefully drawn into a hemocytometer pipet with large bore. Such a pipet may be kept in 80% alcohol and washed before using with sterile citrate. The pipet is shaken for 1 minute, a wide rubber band stretched over its ends and it is then transferred to an incubator at 37.5° C. where it lies for 1 hour in a horizontal position. During this time it is removed and shaken for 1 minute at the end of 15, 30 and 45 minutes.

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[48] Arch. of Int. Med., Jan., 1918, xxi, p. 59.

At the end of the hour the pipet is taken from the incubator, shaken for 5 minutes and cover-glass preparations made in the usual way. To stain this smear it is covered with 2 drops of a polychrome blood stain for 1 minute, then 4 drops of distilled water are added and the diluted stain allowed to remain on it for 2 or 3 minutes. This is washed off with water and the stain differentiated for 10 seconds in 0.02% yellowish water-soluble eosin, washed with water, dried and mounted in colophoniumxylol.

On examination, the cytoplasm of certain of the mononuclear cells is seen to contain many carbon particles while that of the lymphocytes and the majority of the polyphocytes cells contains none. The smears are surprisingly free from extracellular carbon. If it were not for the carbon within these cells some would be mistaken for large lymphocytes although most of them fall in the classes commonly known as transitional and large mononuclear leukocytes.

The average diameter of the mononuclear phagocytic cells closely approximates that of the polymorphonuclear neutrophile but many are smaller than the neutrophils and approach the larger lymphocytes in size while a few are larger than any neutrophile. The cell outline is usually round, but irregularities may be produced when the smear is made, or pseudopodia may have been projected.

The cytoplasm of this class of cells which pick up the carbon particles is characteristic. Considerable lampblack is taken up by all of these cells and many contain so much that the character of the cytoplasm cannot be made out.

The zone of the cytoplasm is wide at some point, due to an indentation or eccentric position of the nucleus. In preparations stained in the usual way this is, next to its phagocytic properties, the most distinguishing feature of the cell. In many the cytoplasm forms only a distinct band about the nucleus but it may be fully as wide as the nucleus itself. The cytoplasm stains a paler blue than that of the lymphocytes. It may be quite free of protoplasmic granules, but many contain granules quite similar to those of the neutrophiles except that they are more filamentous. They are like those of the neutrophils in their oxydase reaction, but are usually less distinct. Typical discrete "azur" granules, such as occur so commonly in lymphocytes, have not been observed.

The nucleus is round, oval, horseshoe or saddle-back in shape, or presents a broken irregular contour. In a few cells of this type the nucleus is stellate or divided into separate chromatin masses. This morphology appear to develop during the hour of incubation, but it is not certain that an occasional cell of this class with a broken nucleus may not be found in the blood. The only important characteristic of the nucleus is that it is very rarely both regular in outline and centrally placed. It stains less heavily than the nucleus of the neutrophiles.

Polymorphonuclear Neutrophiles (Plate I, 6, 7).—These cells which make up from 70 to 72% (Miller 64.2%) of the leucocytes of the adult and from 18 to 40% of the child's are about 10μ in diameter although in a well-spread smear, in which case they have flattened out upon the glass, they may seem about twice this size. The nucleus is characterized by its polymorphous nature and its deep homogeneous stain. It may be a strand variously bent, or 2 or more small fragments connected by fine filaments. The protoplasm takes a faint acid stain and is well filled with the neutrophile granules. These cells are the ordinary pus-cells of inflammatory exudates. They sometimes contain glycogen.

Eosinophiles (Plate I, 2).—These cells are of the same size or perhaps a little larger than the finely granular leucocytes which they resemble in every way except that their protoplasm is often slightly more abundant and is filled with eosinophilic granules. These cells make up from 2 to 4% (Miller 2.8%) of the normal leucocyte count.

Mastzellen (Plate I, 8).—This name is given, perhaps incorrectly, to any cell with basophilic granules. These cells are usually about the same size as the preceding but more often are smaller. Their nucleus is polymorphous, very faintly staining and often trilobed. The protoplasm contains a variable number of granules of different sizes, yet for the most part as large as α granules, which form a band around the nucleus. These granules are not stained by the triple stain, hence one sees only the spaces which they occupy. (These are probably the reticulated or vacuolated cells of Uskow.) They stain best in thionin and are said to take a metachromatic tone. These cells make up about 0.5% (Miller 0.6%) of the total count.

In addition to these the following leucocytes may be found in pathological conditions:

Myelocytes (Plate I, 9, 11, 14, 17).—While any cell of the bone-marrow is, strictly speaking, a myelocyte, by this term is generally meant 1 with granular protoplasm and a round nucleus. Some are neutrophilic, some eosinophilic, while some claim to have seen true basophilic myelocytes. *Neutrophile Myelocytes.*—The size of these cells varies from that of the large mononuclears to that of red corpuscles. The largest and smallest neutrophile myelocytes are found in the blood only in myelogenous leukemia, but a few neutrophilic myelocytes the size of the granular leucocytes may be found in any condition with a high leucocyte count. The characteristic point is the shape of their nucleus which is either perfectly round, oval, indented or kidney-shaped, but never polymorphous or pycnotic; if it were, the cell would count as an ordinary leucocyte. It is usually centrally placed. It is impossible to draw a sharp line between a myelocyte and a polymorphonuclear cell (Plate I, 12, 13) since every possible gradation occurs, but as myelocytes we count all granulated cells with round, oval, or kidney-shaped faintly staining nuclei, providing the nucleus apparently occupies at least ½ of the cell. A cell with nucleus relatively smaller, more compact, more distorted and more diffusely stained ranks as a leucocyte. The nucleus of some leucocytes is round or oval but it also is relatively small (occupying only about a quarter of the diameter of the cell) and stains deeply and diffusely. The chances are that could we get a side view of these nuclei we would find them polymorphous. For the question of the motility of myelocytes, and most now agree they are ameboid, see the writings of Wolff.[49]

Some myelocytes are full of granules while others have but few and these are scattered. The very large forms are met with in the bone-marrow and in well-made specimens of blood of cases of myelogenous leukemia, but as a rule one sees only a large faint nucleus surrounded by granules free in the plasma.

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[49] Deut. med. Wochenschr., March 5, 1903.

Eosinophile Myelocytes (Plate I, 14).—The coarsely granular myelocytes are the exact analogue of the preceding and occur under much the same conditions, but less often and in much smaller numbers. They are found especially in splenomyelogenous leukemia and in anemia pseudolymphatica infantum.

Small Neutrophiles: *Pseudolymphocytes.*—The small neutrophile leucocytes have a round, intensely staining nucleus and a narrow margin of protoplasm full of neutrophile granules. Their size is about that of a lymphocyte. They are rare, occurring especially in pleuritic exudates and are supposed to rise from fragmentation of the polymorphonuclear cells.

Irritation Forms.—The so-called " irritation forms " vary in size from a lymphocyte to a large mononuclear, but the majority are small. Their nucleus is round, of a bluish-green color (Ehrlich stain), often eccentric and has no chromatin net-work. Their protoplasm stains an intense rich brown and has no granules. Türk says that they occur under the same conditions and have the same significance as myelocytes.

Differential Counting.—In making a differential count one first must agree on certain rules of classification which are more definite than our actual knowledge would justify. Since we know so little of the relationship between the various forms of leucocytes, their ages, their origin and of their function, the only classification possible is a purely morphological one. We have rules which should be followed mechanically if our results are to be at all comparable. We separate first granular and non-granular cells. This in a well-stained specimen should be easy. There is no difficulty in separating the coarsely granular and the finely granular cells. Whether or not we are justified in separating the coarsely granular cells into two groups, the eosinophiles and Mastzellen, using specimens stained with methylene blue, is an open question. We do, however, make a separate classification for the Mastzellen.

In normal blood it is very easy to distinguish between the small and the large mononuclears but in some pathological conditions in which large lymphocytes and small endothelial leucocytes are present we must fall back on the unsatisfactory rule that lymphocytes are smaller and large mononuclears are larger than a polymorphonuclear neutrophile leucocyte. For normal blood this classification is satisfactory but in pathological conditions many objections arise. While the lymphocytes seem to form a fairly distinct class although some are large (Plate I; 3, 4, 15, 20), the group of endothelial leucocytes contains large, medium and small forms and any line based on size which divides this group is arbitrary and increases the number of the lymphocytes by cells which do not belong there and diminishes a group which should not be divided. This is best seen in typhoid fever and malaria, diseases in which the group of endothelial leucocytes is increased and this group then includes large and small forms. In the so-called lymphatic leukemia also the small mononuclear cells are certainly not all lymphocytes.

Neither can we separate groups on the basis of an indentation of the nucleus. Ehrlich's name " transitional " is still used although be abandoned it as soon as he discovered that the granular myelocyte and not the large mononuclears with indented nuclei, the so-called " transitionals," were the young forms of polymorphonuclear granular cells.

The line between myelocyte and leucocyte is very hard to draw (see page 498).

In leukemia it is very hard to draw a line between those large mononuclears (Plate I, 16, 19, 21) with deep staining protoplasm and the granular myelocytes and perhaps no such line exists. Yet in well stained specimens one is in doubt concerning but few cells.

One group of cells is confusing, those represented merely by a faint mass of stain. Such cells should be counted as " undetermined cells " for only in that way will the percentage of those groups which are more resistant be fairly correct. These undetermined cells are almost all non-granular small mononuclears.

There should theoretically be little difficulty in differentiating eosinophiles and neutrophiles and yet even in well-stained specimens the question may be so hard that it leads one to doubt the specificity of granules. We believe that now most observers no longer use Ehrlich's stain and so do not look for the characteristic lilac tint of the neutrophile granules, but consider all finely granular cells as neutrophiles and all coarsely granular ones as eosinophiles.

Ehrlich's neutrophilic granulation has not gained the clinical importance which he anticipated. It certainly would be fairer to use the term " finely granular cells " or " fine acidophilic," in case other stains are used and to reserve the term neutrophilic for cells stained with his triple stain, for that is the only successful specific stain we have for those granules.

For differential counting the best of specimens are none too good and far too much time is spent over smears which should be thrown away. We still prefer specimens stained with Ehrlich's triple stain but use those stained with Hasting's stain. Nucleated reds are counted at the same time as the white cells and calculated as " number per cu.cm."

The list of types recognized is, therefore, the following: Small mononuclear (s. m.) large mononuclear and transitional (l.m. & tr.); polymorphonuclear neutrophile (pmn. ε); polymorphonuclear eosinophile (pmn. α); Mastzell (Mastz.); neutrophile myelocyte (myeloc. ε); eosinophile myelocyte (myelloc. α). Nucleated reds; normoblasts (normobl.), intermediates (intermed.) and megaloblasts (megalobl.).

To make a differential count a mechanical stage should be used and at least 500 leucocytes counted. Some keep count with a pencil and paper, 1 column for each group; others use a slide-box divided into compartments by slides, into which he drops beans, one bean for a cell. Since one can start with exactly 500 beans the mathematics of this calculation is easy.

BONE-MARROW

The careful study of the bone-marrow should be required of each student. In it are found normally practically every cell which ever occurs in the blood and a complete series of transitional forms between the apparently related groups.

The study of marrow while fresh is especially valuable, especially that of the ribs of young babies or premature infants, but fragments of ribs removed for empyema and aa autopsy will suffice if fresh enough. A small piece of rib is squeezed in a pair of forceps and a drop of the exuding marrow picked up on a cover-glass and at once pressed down onto a slide. Very rapid work is necessary, since the drop dries very rapidly. It is surprising how quickly some of the interesting mononuclear forms, the " young " cells, disintegrate. The large myelocytes also soon disappear and in leukemia the marrow smear may soon show only a confused mass of nuclei in a cloud of free granules. For stained specimens, the stroke method is best; that is, the marrow is smeared in lines on the cover-glass by drawing this across the end of the bone. The smear is allowed to dry in the air and is then fixed and stained just as are blood smears. If the marrow is fatty the smears do not stain well. If they are to be fixed by heat they should be overheated, best on the copper plate, smear side up, at the spheroidal point (that is, the point at which the drop of water does not boil but merely rolls off the plate) for 45 or more seconds. Such specimens will have some good areas for study, especially at the edges of the thick portions, and a few such fields are all that is desired. Thin well-spread specimens are usually failures. Better a specimen generally too thick but with some thinner areas.

The bone-marrow of certain vertebrates would seem to be made up of a mosaic of little separate masses of different tissues, each concerned with the production of 1 type of cell. These islands cannot be demonstrated in human marrow and yet this would seem to contain quite distinct tissues more diffusely arranged. In some places will be found nests of nucleated reds in enormous numbers; in other places nests of leucocytes, myelocytes and of intermediate forms. Different parts of the same rib vary much, as we have found to be the case in infant marrow. Since the marrow varies so in different bones and in different parts of the same bone, it is impossible from a limited search to say what is the general medullary condition of a given case (Grawitz). This may explain the lack of evident relation between a marrow and a blood picture.

NUCLEATED RED—Blood-Cells. By the term "erythroblast" most writers mean any nucleated red blood-cell. A few, however, use it of a hypothetical colorless ancestral form of these. A better term for these would be " hemoblast."

(1) NORMOBLASTS.—(*a*) *Howell's Mature Nucleated Reds* (Plate I, 29).—These cells have the color of the non-nucleated red blood-corpuscles. Their nucleus is 3μ or slightly less in diameter, is sharply defined and is pycnotic; that is, it is dense, homogeneous, structureless (triple stain), takes a dense uniform blackish-green color, has no chromatin net-work and is often vacuolated, hence often has a bright spot in the fresh and an unstained area in the stained specimen. They often present amitotic figures, *i.e.*, rosette forms of 2 to 4 or even 12 fragments connected by strands of chromatin (see Plate I, 34; Fig. 113, *c*). The nucleus is often surrounded by a clear zone. These nuclei are so characteristic that they may be recognized even if not surrounded by protoplasm, which is often the case since many are found resting upon a margin of the red cell or even at some distance from it. This is due, said Ehrlich, to the centrifugal force which throws the heavy nucleus out of its cell when the specimen is made. Yet this cannot explain all the free nuclei which are seen in specimens made in various ways and

in cut sections as well. Pappenheim and Israel claim that in leukemia especially such free nuclei result from the degeneration of their surrounding protoplasm.

(*b*) *Howell's Immature Nucleated Reds* (Plate I, 30; Fig. 113, *a*).—The immature nucleated reds are a little larger than an ordinary red blood-cell, their color is perhaps a little paler and their nucleus slightly larger, than that of the mature form. They have definite chromatin fibers arranged radially (in leucocytes they form a meshwork) while mitotic figures are not rare. The division of these cells is rapid, requiring but 15 minutes. In the bone-marrow this is the dominant red cell. Between these 2 cells, the mature and the immature, one finds all intermediate stages.

These 2, and all intermediate, forms of nucleated red cells are generally called normoblasts, although the larger immature forms are by some classed as intermediates. They are the precursors of the non-nucleated red blood-cell but do not reach the circulation of a normal adult except as an anomaly. Their appearance in the general circulation indicates an increased activity of the bone-marrow. Many are met with in the blood in pernicious anemia, more in splenomyelogenous leukemia and some in post-hemorrhagic anemias. They appear in the blood of a child more readily than in that of an adult.

These cells normally are " orthochromatic "; that is, they stain like the ordinary non-nucleated reds (*i.e.*, are oxyphilic), but some are fuchsinophilic while others are basophilic (polychromatophilic).

Blood crisis is the name given by v. Noörden to the appearance in the circulation of enormous numbers of nucleated reds and an increase in the leucocyte count, which occurs usually during the convalescence for an anemia. They remain in the circulation for a few days after which there usually is a sudden rise of the red blood-count. The nucleated reds then slowly disappear and the increase in the count is much less rapid until perhaps another crisis occurs. V. Noörden reported cases with gains in the counts of half a million cells in 4 days. Blood crises are supposed to indicate a temporary increase in the activity of the bone-marrow. They are, however, not always a sign of improvement (see page 608) but always indicate a struggle which may prove unavailing.

Intermediate Red Blood-Cells (Plate I, 31).—By the term intermediate nucleated red blood-cell is meant one which is not quite large enough to be called a megaloblast and yet which is definitely larger than a normoblast. Some are large cells with the nucleus of an immature red; others are smaller cells with a reticular nucleus larger than that of a normoblast. If one systematically measures all nucleated reds in a specimen he will find very few of these cells unless one includes the immature nucleated reds of Howell (Fig. 113, *b*).

Megaloblasts.—In the marrow are always found nucleated reds (Fig. 113, f) which are from 2 to 4 times the size of an ordinary red blood-cell. They are round or oval, their protoplasm is abundant and often polychromatophilic and their nucleus is large plump, round or oval, centrally placed and conspicuous, while in stained specimens a good chromatin net-work is demonstrable. These cells are quite similar to the large nucleated reds seen in primary anemias and called megaloblasts.

To understand a writer's report of a blood case it is necessary to know his definition of a megaloblast. Opinions vary much. Some demand that the cell be large, others that it have a large nucleus. Our rule is that both of these specifications must be fulfilled, and, for reasons to be given later we ask that the size of the nucleus shall be at least that of an ordinary non-nucleated red (7.5μ). Pappenheim and others consider the megaloblast a different type of cell from the normoblast and say that it may be recognized by certain fine points in the nucleus even though the cell be as small as a normoblast.

The megaloblast of the circulating blood in pernicious anemia differs somewhat from the above mentioned large nucleated red cell of the bone-marrow. They are about equal in size but the normal bone-marrow megaloblast usually has a round nucleus with a very distinct margin and a definite chromatin net-work, while the nucleus of the megaloblast in the blood in pernicious anemia often is more oval, much less distinct, stains much fainter and has less definite nuclear membrane and chromatin structure. But these differences are slight and inconstant.

The significance of megaloblasts in the blood has been the subject of much controversy. Ehrlich claimed that they are never found in the normal bone-marrow of an adult, but rather are the product of a megaloblastic degeneration of this tissue due to a toxine which brings about a reversion of the marrow to the embryonic (others say " to the amphibian ") condition. Ehrlich well said that any attempt to break down the distinction between normoblasts and megaloblasts fails from the fact that in pernicious anemia the entire blood picture is megaloblastic. (We are inclined to think that the expression " reversion to the amphibian type of blood " is much too often used. The only amphibian we have studied whose blood resembles that of pernicious anemia is the batrachoseps, and one would hardly call an hemoglobinemia a " reversion," although that is the blood condition in some worms). While a " megolablastic degeneration " may explain the large numbers of megaloblasts in the marrow of cases with pernicious anemia and in some other conditions, we fail to find any recent observer who has not found them in all normal marrows. In our studies of bone-marrow we have measured many nucleated reds and have found the predominant cell the immature normoblast, with a nucleus between 3 and 4μ in diameter. The next most common cell, about 15% of all nucleated reds, is a megaloblast, with a nucleus of 7μ or over in diameter, or 7μ in 1 axis if oval. Between these 2 groups of cells occur every intermediate size, and yet their number is not as great as that of the large cells.

Bunting has shown that in certain animals the marrow contains islands of nucleated reds in the center of which are megaloblasts surrounded by zones of cells of different size until one reaches to the periphery where normoblasts are found. The activity of these zones produces cells of the same size as well as those for the layer external to it. Ordinarily the cells for the circulation are produced at the periphery of these islands but if the islands are over-taxed or affected by certain poisons the successive layers are in part stripped off and larger cells of the interior may send cells into the general circulation. This could be a very satisfactory explanation of the megolablastic character of the blood in pernicious anemia and in the bothriocephalus anemia. It might also explain the blood crises, etc.

Karyokinesis of these large cells occurs in the peripheral blood in severe anemias, especially as a terminal phenomenon.

Thayer has seen definite ameboid movements in a megaloblast.

Microblasts.—By microblast is meant a nucleated red under 6μ in diameter with a small pycnotic nucleus. They occur in the circulation in severe traumatic anemias, leukemia, etc. Some appear to be perfect cells and these may be the forerunners of microcytes, but others would seem to be pinched off from larger cells.

The fate of the nucleus of the red cell is still the subject of much discussion. Two views have been held: (1) that the mature normoblast extrudes its nucleus (Rindfleisch, Howell, *e.g.*), and (2) that it disappears within the cell by karyorrhexis and karyolysis (Kolliker, Neumann, *e.g.*). Those who hold the latter opinion admit that some nucleated red cells may be seen to extrude their nuclei, but consider it pathological. Other writers believe that all of these methods are possible; Ehrlich, for instance, thought that the normoblastic nucleus is extruded but the macroblastic is absorbed. In favor of the extrusion theory is the fact that most non-nucleated red cells are flat, disk-shaped and even biconcave. But some are even spherical, especially in the embryo, a point emphasized by those holding the theory of absorption. The " degenerations " or nuclear fragments described by Vaughan (page 449) and by Cabot (page 477) would suggest karyorrhexis. The free nuclei so often seen in stained specimens may have been thrown out of the normoblasts by the centrifugal force caused by the sudden motion of the cells when the specimen is made and the reason why the nucleus of the megaloblast remains in the cell may be that its specific gravity is nearer that of the protoplasm.

The changes in the nuclei are important. By a "pycnotic" nucleus is meant 1 diminished in size, dense, homogeneous, sharply defined, sometimes vacuolated and without any evident chromatin net-work, all of which suggest a solution of the chromatin in the nuclear fluid. Pycnosis may be a preliminary step of karyolysis or absorption although in the latter case the nucleus as a rule becomes fainter till it cannot be distinguished from the surrounding protoplasm. Pycnosis may precede karyorrhexis or fragmentation of the nucleus, which fragments may then disappear by karyolysis. The normoblastic nucleus may by amitosis divide into polymorphous forms with 2 or even 12 fragments (Plate I, 34) of equal or unequal size and usually united by a filament. In 1 of our cases during a blood crisis 55% of the erythroblasts were of this description. Another method of nuclear destruction is suggested by cells containing only a few chromatin strains and masses as though nuclear membrane and fluid disappear first.

A point evident in bone-marrow specimens is the varying depth of the hemoglobin tint of the red corpuscles, a variation much greater than is seen in the blood. This might suggest that the formation of hemoglobin is a gradual intracellular process. This is a far-reaching problem not only in cytology but also in diagnosis (see page 592). The question is of particular interest in the study of the anemias. Are these pale cells in the circulation permanently pale or are they only immature and later develop more hemoglobin? When the color-index falls is it because cells are losing hemoglobin or because new light-weight cells are replacing heavier ones? As the case improves do cells develop more hemoglobin? That is, is the cell like a coal cart which carries different loads at different times? Arguments from comparative anatomy are not satisfactory, yet the red cells of some of the lower vertebrates would seem to complete their development in the circulation. In the mammals an imperfect cell is said to be incapable of further development. Finally, Gaule and his pupils believe in a hemoglobin "store" in the body, which hemoglobin in case of need is carried into the circulation in new corpuscles and returned when the extra cells are withdrawn. In adult man the evidence would tend to show that a red cell is the product of erythroblastic tissue not of hemoglobin free tissue, that each cell in the circulation whether complete or incomplete is finished so far as that cell is concerned; that the formation of hemoglobin is a slow process, much slower than is the proliferation of new cells; and that the bone-marrow, when the demand for new cells is heavy, uses its available store of pigment to make light-weight cells and later replaces them with cells of normal weight. This is reasonable since red cells are functionally valuable in proportion to their surface, not their weight.

ORIGIN OF RED BLOOD-CELLS.—That the ordinary non-nucleated red blood-cells come from nucleated reds is now doubted by few. In the embryos and possibly later under conditions of increased hematopoiesis there is also the intracellular differentiation of erythrocytes within the hemogenic polykaryocytes (see page 506).[50] Up to the end of the fourth week of embryonic life all of the blood-cells are nucleated. From that time on the number of the non-nucleated cells increases so that at the third month only about ⅛ to ⅙ are nucleated. At the fifth month nucleated reds are still numerous but at birth it is seldom that one is found in the blood.

In the earliest embryonic life the blood-vessels are formed from solid cords of cells the peripheral cells of which become the endothelial lining of the vessel wall, the internal cells the corpuscles. This process may occur in almost any part of the developing organism and may also in the adult wherever new blood-vessels are formed. In the embryo also many mitoses are found in the nucleated reds of the circulating blood.

Before the third month the liver has become the chief seat of blood formation, after the fifth month the spleen and the lymph-glands take up the task but finally the marrow becomes the chief blood-building organ. In the child the marrow of the whole skeleton has this function but at about puberty and during adult life only the ribs and some of the flat bones. Howell considers that callus, for instance that following a fracture, may in the adult for a while furnish centers for hematogenesis. In the adult it would seem as if the spleen could resume this function in leukemia and anemia.

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[50] Jordan Am. J. of Anat., 1918, Vol. 24, p. 225.

Removal of the spleen causes very little anemia, but about a month later the small mononuclears begin to increase and this continues for months resulting sometimes in a blood picture which strongly suggests leukemia. About 12 months after the splenectomy an eosinophilia of even extreme degree may develop. These phenomena are now explained as evidence of the vicarious activity for the spleen, first by the lymph-glands and then by the bone-marrow. In health one of the functions of the spleen seems to be to remove old and injured red cells and leucocytes from the circulation and the acute spleen tumor in some conditions may be due to the great number of leucocytes ingested (spodogenic splenic tumor).

In the embryo the blood-cells at first are without hemoglobin. At this time also there are no true leucocytes and none appear until after the formation of hemoglobin-containing cells is quite active. The embryologists have shown that in disease of the embryo before the appearance of leucocytes the red blood-cells are ameboid and perhaps phagocytic, which is interesting since in certain blood diseases of the adult one gets hints at least of these 2 functions.

The view is still held by some that in some forms of acute lymphatic leukemia the abnormal white cells are " red cells " without hemoglobin, and the converse of this also is held by some to be true, that is, that in severe anemias some of the large lymphocytes develop to megaloblasts instead of to small lymphocytes (Pappenheim).

Howell was the first to demonstrate in the cat the " ancestral red corpuscles " which resemble the red blood-cells of reptiles since they are large, oval, semifluid red cells, with a deeply stained oval nucleus. These cells were later described by Engel as " metrocytes of the second generation," those of the first generation having a large chromatin-rich nucleus. Such cells later never, normally at least, reach the circulation and Engel thinks they are no longer formed.

For the study of the young red cells the blood of embryo mice is to be especially recommended. Here a great variety of changes in the nucleus and of granulation of the protoplasm may be demonstrated.

White Blood-Cells.—In the marrow one finds: *A*. GRANULAR CELLS. I. NEUTROPHILES. *Neutrophile myelocytes.*

1. Typical myelocytes are cells from 12 to 15μ in diameter, with a large round nucleus and protoplasm scanty and finely granular which forms often a thin rim around the nucleus. These cells are by far the most numerous in the marrow but on account of their size seem even more so than is the case. The nucleus is often hard to make out. In the bone-marrow may be seen also the much larger beautiful myelocytes with faint nuclei, " Cornil's marrow cell," which however are not often seen intact since they disintegrate so readily. These appear in the blood only in leukemia. In the fresh marrow some of the myelocytes have a very small, dense, round nucleus, which we think is due to post-mortem loss of nuclear fluid. All transitions from typical myelocytes with round nuclei to typical leucocytes are present on the one side (these transitional forms are called " Netamyelocytes ") and all transitions from typical myelocytes full of granules to large mononuclears with clear protoplasm (myeloblasts) on the other. The transitional forms between myeloblasts and myelocytes are called " promyelocytes."

2. Cells similar to the above, but much smaller, their nucleus indented, or slightly polymorphous and staining faintly, are called *transitional cells* (between 1 and 3).

3. Typical leucocytes.

II. EOSINOPHILES.—These always are relatively few in number.

1. Eosinophile myelocytes. Large cells with pale nuclei, scanty protoplasm filled

with eosinophile granules, otherwise similar to the above mentioned neutrophile cells.

2. Similar to the above, but smaller; the nucleus indented or slightly polymorphous. All gradations are seen from this to typical—

3. Eosinophile leucocytes.

III. BASOPHILES.

1. Mastzellen, which, however, are rather rare. The nuclei of these cells may have a variety of shapes, yet are usually polymorphous (see page 498). Mononuclear Mastzellen occur (Engel). These are, at least, hard to recognize, since the young (?) α and ϵ granules are quite basophilic.

2. Cells varying in size but the most of them small and containing violet colored granules with basic stains. These, although they contain basophile granules, are not typical Mastzellen. In this connection it should be mentioned that the β granules may occur in the eosinophile cells or by themselves.

3. Polymorphous cells, with fine basophile granules. These may be neutrophile cells stained by the " tricky " methylene blue.

B. NON-GRANULAR CELLS.—1. Lymphocytes. These have the size of red blood-cells, a narrow rim of protoplasm and a nucleus rich in chromatin. These cells are the second most numerous cells of the marrow and often resemble naked nuclei.

Among these are the " protoleucocytes " of Osler; solid-looking lymphoid elements from 2.5 to 5μ in diameter, which resemble free nuclei; some have a rim of protoplasm. From these " erythroblasts (?) " develop.

2. Medium-sized lymphocytes with more protoplasm and a smaller and often eccentric nucleus.

3. Very large cells with general character of lymphocytes which appear in the blood in some acute leukemias, but never normally. These are the " Large lymphocyte " (Ehrlich, Fränkel, Pappenheim); Grawitz's " unripe cell," Wolff's " indifferent lymphoid cell," Naegeli's " myeloblast," Troje's " marrow-cell." Their nucleus stains faintly, is seldom lobulated, is very pale and poor in chromatin and their protoplasm is faintly basophilic.

Also to be mentioned are cells, common enough, which in the fresh marrow resemble normoblasts (immature), except they have no hemoglobin. They are from 9 to 12μ in diameter. Their nucleus is that of Howell's immature red (see page 502), and they have a hyaline protoplasm. These are the " erythroblasts " of Osler, Löwit, and Howell.

Löwit described as " leucoblasts " cells with relatively large nuclei which contain 1 or 2 chromatin masses which are sometimes irregular in shape and from which a system of delicate lines and bands radiate to the distinctly doubly contoured nuclear membrane which has on its inner surface projections connected with the infranuclear net-work.

Ehrlich believed the true lymphocyte came from the lymph-glands; others say only from the bone-marrow. Some have tried to distinguish morphologically those from the marrow from those from glands (Rubenstein). The question now is, " Do any come from lymph-glands? " thus admitting that typical " lymphocytes " are an important constituent cell of the marrow. Michaelis and Wolff tried to differentiate these cells on the basis of their future history, the lymphocytes from lymph-glands remaining such, while the " lymphoid " cells of the marrow were capable of further development to a granular cell. But this " capability " would be hard to determine in the case of the individual dead cell now before us, although these writers did also describe slight differences in their staining reactions. And yet a distinction between lymphocytes and lymphoid cells is probably quite just (see page 469).

Many workers considered these lymphoid cells as young forms and named them " protoleucocytes " (Osler), etc., considering that from them develop the colorless cells which correspond to the leucoblast and the erythroblast of Löwit and Howell, from which develop the whole series of the red and the white cells. The lymphocyte with diffusely staining notched nucleus (Rieder's cell) is probably an old form of lymphocyte.

The small mononuclears of the bone-marrow with round vesicular nucleus, delicate chromatin net-work and rather broad band of basophilic protoplasm with smooth margin, are young cells which resemble those of the normal blood only in appearance but themselves do not belong there. The lymphocytosis of young babies, the most rational explanation of which is an overproduction of leucocytes to fulfill a function which did not formerly exist, would suggest that young lymphocytes are lymphoid cells.

In the marrow are cells which never reach the normal circulation. Among these are young (also old?) leucocytes and red blood-cells and some say the undifferentiated cells which are the ancestors of both series, although we follow Bizzozero that none of the ancestors of red cells are without hemoglobin. The trouble is that there is such a variety of cells found in the marrow that one may find evidence in favor of any ancestral tree he wishes to draw. Formerly the series were traced back to small cells but now the cells considered young are very large, with large, faintly staining nuclei and protoplasm which very quickly goes to pieces, hence they are seldom seen. Probably too much stress has been laid on the shape of the nucleus as evidence of age. It may be that irregularity in the nucleus is merely an expression of function.

Most will agree that there is in the marrow a large group of indifferent cells which may develop in some direction or other, possibly to red or white series as necessity demands. The only question is, " Which are these cells? "

The development of cells is more by " steps " than by a gradual transition and those of each step are able to produce others of their kind as well as some of the succeeding generation. The picture as usually drawn is very complicated, since the line of descent of these cells is not single but several ancestors may produce the same forms so that to trace the description backward is more like following a stream toward its source. It is pictured as a single river at its mouth, but as we go toward its source many tributaries are found which contribute to its volume. Thus Pappenheim says normoblasts come from small lymphocytes, megaloblasts from large lymphocytes, and considers the polychromatophilic group as evidence of the transformation from a basophilic lymphocyte to a red cell; subsequent workers trace normoblasts from megaloblasts. Normoblasts certainly can produce normoblasts and megaloblasts, megaloblasts. Again the granulation may appear in cells with nuclei at various stages of deformity as if the changes in the nucleus from round to polymorphous bore little parallelism to the development of granules.

We cannot here take up the question of the origin in other organs of leucocytes and perhaps of red cells. The above remarks are not intended as a resume of the subject, but an answer to many of the questions asked by students while studying the smears of bone-marrow. We will merely mention Nothnagel's case of general osteosclerosis with the entire marrow practically functionless, yet with a normal count of neutrophiles; also the presence of mononuclear granular cells in areas of inflammatory infiltration.

4. Pigmented cells, often absent.

5. Giant-cells.[51]—(*a*) Megakaryocytes with 1 large irregularly coiled nucleus. These " giant-cells with budding nuclei " are considered as the parent cells of the blood platelets.[52] Jordan believed that the megakaryocytes (polymorphokaryocytes) are hypertrophied hemoblasts; that their basket nucleus undergoes direct division producing polykaryocytes which are hemogenic giant cells. These are essentially multiple hemoblasts comparable to the blood-islands of the yolk-sac and under certain conditions, apparently those of increased hematopoietic activity, may differentiate erythrocytes intracellularly. Jordan suggests that these cells represent an incidental phase of intense hematopoiesis; he grants that certain specialized megakaryocytes may serve as sources of origin of blood platelets (according to the conclusion of Wright).

Jordan does not believe that these cells are phagocytic.

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[51] Jordan Am. J. of Anat., 1918, xxiv, p. 225.

[52] Wright Jour. Morph., xxi, p. 265.

(*b*) Giant phagocytes, always mononuclear and resembling large lymphocytes or endothelial leucocytes, their giant size due to their swollen condition from the large number of ingested erythrocytes. These cells are seldom found in the stained specimen although one does find masses of detritus which may represent them. These cells may enter the circulation in cases with marked leucocytosis and are filtered out in the lung (see Plate II, 11).

(*c*) Multinuclear osteoclasts which are not related to hemoblastic polykaryocytes. These also are polynuclear.

Many cells are seen, especially in fresh specimens, which contain very interesting degenerations and inclusions. Some large mononuclear cells contain large globules or droplets about 3μ in diameter which are rather uniform in size and have the yellowish shimmer of the myelin droplets of the sputum. Some cells are filled with very large granules which have the color and refractility of α granules (see Fig. 115, *e*). Howell found many such cells in the marrow of the cat and thought that they must play an important part in metabolic changes in the marrow. In other cells one sees globules of fluid which give them a vacuolated appearance. In some cells large " dropsical " projections both of protoplasm and of the nucleus are seen.

It is much easier to trace the degenerations of the leucocytes than of the red cells. In normal blood practically all the leucocytes are normal, but when there is a leucocytosis and especially in the leukemias one finds many cells with definite degenerations. The lymphocytes may be almost devoid of protoplasm, their nucleus is small, pycnotic and indented, or even polymorphous (Rieder's cells) (Plate II, 17).

The nuclei of the polymorphonuclear granular leucocytes are very pycnotic and fragmented although probably all the fragments are corrected by a chromatin thread. For an interesting classification of the neutrophile leucocytes based on the number of nuclear fragments, and the clinical use to which such a classification may be put, see the publications of Arneith.[53]

The large pale nuclei without protoplasm seen in the marrow are said to be the nuclei of very sensitive young cells the protoplasm of which is destroyed in the preparation of the specimen, but similar appearances in lymphatic leukemia suggest very strongly that these may be only degenerated lymphocytes.

Late in leukemia it would seem (said Ehrlich) as if the ability of the marrow to develop neutrophile granules might be lost, and clear cells are found which resemble the granular cells in every way except that their protoplasm is clear.

Do the blood leucocytes have the ephemeral history which some ascribe to them? Winternitz (quoted from Grawitz) estimated that in the dog the lymph supplied the blood stream daily through the lymph-duct with a number of lymphocytes equal to more than half the total number in the body at any one time. If this is true, then the chief function of most of the white cells must be to increase the proteid content of the plasma. A similar question arises in cases with profuse pus formation, as cystitis, bronchitis or bronchiectasis, since some of these patients are estimated to lose daily a number of white cells almost equal to the total number in their circulation at any one time.

Fetal Blood.—In the 3-months' human embryo Engel found nucleated red cells of normal size and others larger, the " metrocytes of II Generation." These latter cells he describes as large spherical nucleated reds, 12 to 20μ in diameter, rich in protoplasm and with a relatively small nucleus 3.5 to 6μ in diameter. (But some of these cells measured from 17 to 20μ in size and had a nucleus measuring from 7 to 8μ.) These numbered from 4 to 6 per 100 normal reds. (Metrocytes of I Generation he describes from mouse embryo's blood as spherical cells from 2 to 3 times the size of a normal red cell, the nucleus often in mitosis and filling but a relatively small part of the cell; this, he says, is not a megaloblast nor a gigantoblast. At this stage there are no non-nucleated reds and no leucocytes.) At this stage are found 2 forms of normoblasts: those staining orange, from 5 to 9μ in diameter and their nucleus 3.5 to 5μ; and those staining red (Ehrlich stain), about 7 to 8μ in diameter, with a relatively large nucleus rich in structure 5 or 6μ large and their protoplasm scanty and ragged. Belonging to this latter group are some large cells 16μ in diameter and a nucleus of 11μ. These are Ehrlich's megaloblasts.

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[53] Zeitschr. f. klin. Med., 1904, Bd. 54, p. 232.

In embryos of 6 cm. length the non-nucleated reds were to the nucleated as 12 : 1; of 12 cm. embryo, 55 : 1; of 16 cm. 150 : 1; of 19 cm. 176 : 1. In the 6 cm. embryo the metrocytes were 4% of the reds; in the 12 cm., 0.25%, and latter none were found. The leucocytes in the 6 cm. embryo were to the reds as 1 : 500 to 1000.

Engel admits that embryos of the same age differ so that he could not tell the age of the embryo by studying its blood.

We have had opportunity to study the blood (see Fig. 124) of a fetus 15 cm. long, and found: red cells 1,168,000; hemoglobin 25%; leucocytes 9000; nucleated reds 1 : 19 of the total reds; normoblasts and intermediates some showing beautiful polychromatophilia.

In an embryo 20 cm. long we found: reds 2,652,000; leucocytes 28,000; hemoglobin 38%.

In an embryo of 23 cm. Engel found the reds (heart's blood) 3,300,000; hemoglobin 80%; leucocytes 40,000. Nucleated reds were to non-nucleated cells as 1 : 120 and all red cells with nuclei were normoblasts. Of the leucocytes, the granular were to the non-granular as 2 : 5; neutrophile myelocytes and leucocytes and all transitional forms were present; also a few eosinophiles.

The blood of a 27 cm. embryo contained nucleated and non-nucleated reds in relation of 1 : 200, leucocytes to erythrocytes as 1 : 90, polymorphonuclears to mononuclears as 4 : 5.

LEUCOCYTOSIS

By the term leucocytosis was formerly meant any increase of the white cells of the blood above the highest limit of normal, that is above 10,000 per cmm. As the term is now used, however, the increase must be transitory and it must affect especially the polymorphonuclear finely granular cells. Ten thousand leucocytes per cubic milimeter may be considered the upper limit of normal although some normal persons have for a long time a leucocyte count of from 10,000 to 12,000. Yet in judging a leucocytosis we consider as limit more the count which would be expected in that particular person at that particular time; that is, in the condition in which he then is. Many cachectic persons have for long periods of time very low counts *e.g.*, 4000 cells, and a rise to 8000 would mean for them as much as a count of 20,000 would in a more normal person, for a leucocytosis represents a reaction, a struggle, and the result must be judged relative to the person making the struggle. One of our cases of typhoid fever had a leucocyte count of 1600. A parotitis developed and the leucocytes promptly rose to 3200. This was a true leucocytosis for that person at that time.

A leucocytosis also is transitory and symtomatic. This distinguishes it from leukemia. And, finally, the term leucocytosis is used if the rise is due to an increase of the polymorphonuclear neutrophile cells. If the increase involves the mononuclear non-granular cells, the term *lymphocytosis* is used; if the polymorphonuclear eosinophiles, *eosinophilia*; if the mononuclear granular cells, *myelemia*, etc. It is very seldom that 1 group of cells only is increased; usually other groups also are, but to a less degree. To some this would suggest that these groups are directly related, but a better explanation may be that several tissues respond to the same stimuli. Since the various cells may have little relationship to each other, that is, since 1 cell is not certainly a younger form of another, it is their absolute number which should be considered rather than their relative number, that is, rather than their percentages or " formula." The absolute number of a group of cells may increase even though its percentage diminishes providing the total count rises, while the reverse also is true that when the percentage seems to indicate an increase the absolute number may have dropped if there is a diminution of the total number. For this reason one should not make a differential count unless he makes a total count at the same time.

That 1 group of cells may remain unchanged while other groups change much, is well illustrated by the case of Frazier and Halloway, in which the count was 13,040. Of these the polymorphonuclears were 78.2% (*i.e.*, 10,197) and the small mononuclears 16.8% (2101). The total count later rose to 54,960 of which the polymorphonuclears were 90.4% (49,684) and the small mononuclears only 4% (*i.e.*, 2198 or the same as before).

The *general type* of leucocytosis—*i.e.*, one with an equal increase of all the types of leucocytes—is rarely seen. Such a leucocytosis does result, *e.g.*, from stasis of blood in the capillaries, following a cold bath, or massage, while the digestive leucocytosis and that of pregnancy suggest this in some degree.

Classification of the Leucocytoses (Limbeck).—1. Physiological: (*a*) Digestion; (*b*) Pregnancy; (*c*) Newborn. 2. Pathological; (*a*) Inflammatory; (*b*) Malignant tumors; (*c*) Post-hemorrhagic; (*d*) Agonal. 3. After medicinal and therapeutic measures. 4. Various other causes, as shock, etc.

Physiological Digestion Leucocytosis.—If following a fast of 12 or more hours a normal person partakes of a meal rich in proteid his leucocyte count usually will rise to about ⅓ above its normal number. The count should be made hourly. It begins to rise in about 1 hour, reaches a maximum in from 3 to 5 hours and then decreases. While the increase involves the polymorphonuclear neutrophiles especially yet the small mononuclears are increased to some extent, in some cases considerably. For some persons a preliminary fast is not necessary; others do not show this leucocytosis at all (Limbeck thinks habitual constipation explains this failure). Children show it more markedly than adults and the well nourished more than the poorly nourished. It is greatest in the infant after his first meal of cow's milk. In the nursing infant it is said to be absent and hence the opinion (Moro) that when it does occur it represents a reaction against a foreign proteid. Since due to proteid diabetics show it well. It is important that the meal be unusually large since the leucocyte count may not change and even may drop after a light meal. This leucocytosis cannot be demonstrated in herbivorous animals and with difficulty in man after a vegetable meal.

This leucocytosis is supposed to be due to the positive chemotactic influence of the absorbed products of proteid digestion. Hofmeister suggests that a proliferation of the large masses of lymphoid tissue along the intestine, which accompanies the digestive processes, may be the cause, and in support of this calls attention to the coincident increase in the mononuclear non-granular cells. This accompanying lymphocytosis is, however, not always present.

Jaffé says that in children the leucocytosis is not dependent on the meal, but is periodic.

The reverse relation is also true. Starving persons have a low leucocyte count. Succi, who fasted seven days, had a count of 861 per cubic millimeter, while the insane with melancholia often have counts below 3000. On the other hand, well-nourished persons often have counts from 10,000 to 12,000. From this it has been argued that the leucocytes play an important part in the absorption,transportation and assimilation of food and so their number will depend much on the age and nutritional condition of the person.

The attempt has been made to use the digestion leucocytosis as an aid in the differential diagnosis between pernicious anemia and cancer of the stomach. In severe blood diseases, as pernicious anemia, in ulcus ventriculi and in other gastric diseases a digestion leucocytosis can be demonstrated, while even in fairly early cases of cancer of the stomach it is sometimes, but not always, absent. It is absent in some benign gastric conditions.[54] Gastric catarrh and involvement of the lymph-glands are given as the explanation for its absence.

Leucocytosis of Pregnancy.—About 75% of women during the last months of pregnancy have a leucocyte count which is above normal, averaging about 13,000 per cubic millimeter. This is especially true of the primipara and yet the explanation may be more her youth and better nutritional condition rather than the fact that she has had no previous pregnancies. The count rises until the end of pregnancy and then diminishes for from 4 to 14 days after delivery. The differential count may remain practically normal and yet the polymorphonuclear neutrophiles are usually increased. In multiparæ the leucocytes rise but usually within physiological limits.

V. Limbeck considers the leucocytosis of pregnancy as a prolonged digestion leucocytosis, due to the need of additional nourishment for the mother and child. In favor of this is the observation that the count is not increased, is in fact even diminished, after a heavy meal. This is due, it is said, to a migration of leucocytes to the placenta where is the greatest accumulation of the positively chemotactic products of digestion. The condition of the breasts is also suspected; others ascribe it to an overactivity of the lymphatic system. But the view most commonly held now is that in pregnancy there is a slight intoxication against which a primapara reacts differently than a multipara. Thomson found that of 33 counts on 12 pregnant women made during the 8 months of pregnancy but 1 was below 7000; the highest was 13,200.

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[54] Renchi, Arch. f. Verdauungskr., Bd. vii.

But the question is, What is the usual count for a normal woman? Is it 5500? If so, pregnancy causes in all cases a relative rise and in most an absolute leucocytosis. Zangemeister and Wagner [55] think the question a complicated one. Of 47 normal non-pregnant women, from 21 to 34 years of age and all under practically the same conditions, 35 (74%) had counts above 10,000 (mean count about 12,500). The leucocyte counts of pregnant women (57 cases) varied within the same limits as the non-pregnant (70% above 10,000; mean count between 12,500 and 15,000), nor did the number of previous pregnancies seem to make any difference. During labor the count rose in nearly all of the 63 women examined even to 3 times the previous count, the maximum at or just after delivery. This was especially marked in cases of prolonged labor and of those who suffered greatly. In quick, easy labors the rise is insignificant.

In 75 cases the count decreased during the puerperium rapidly to normal. On the seventh or eighth day there was an increase of mononuclears which accompanied the involution of the uterus. (Rouslacroix and Benoit). A study of 2 cases of version led them to think that the rise might be due to the contractions of the uterus.

In pathological cases the leucocytes give no aid in diagnosis or prognosis, since the counts are no higher than some seen in the physiological cases.

Lobenstein [56] believes that there is a genuine leucocytosis of pregnancy. He found that the average of 50 cases during the ninth month of pregnancy was 11,854 for primiparæ and 9346 for multiparæ; and on the third day of the puerperium 13,200 for primiparæ and 11,600 for multiparæ. These figures are too nearly normal to have great significance. The digestion leucocytosis test was tried in 20 cases and found present (p. 510) in 13 while an actual diminution in the count was noted in 6. Of 13 cases of eclampsia, in 6 mild cases the highest count was 31,000; in 6 severe cases from 40,000 to 50,000; and in 1 severe fatal case it was 106,000. He concludes that the leucocytosis runs roughly parallel to the degree of intoxication and to the resistance. A low count and a rapidly falling count are bad signs.

LEUCOCYTOSIS OF THE NEWBORN.—Although the fetus has many blood-building organs yet the leucocyte count is very low since there is as yet no function for these cells (Askanazy). The statement is often made that at birth the leucocyte count ranges from 17,000 to 21,000 and that after the first feeding it rises from 26,000 to 36,000, the increase involving the small mononuclears especially. On the first day after birth Gundobin, Carstanjen, and Warfield[57] found the average count about 26,000 (11,700 to 34,700), while on the third day the average was 13,270 and on the eleventh day 15,740. For the first few days there is an absolute increase in the number of polymorphonuclear neutrophiles. Their percentage was 70.42 on the first day, 53.16 on the third and 34.2 on the eleventh. The large mononuclears and transitionals are high, being 10.76%,

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[55] Deut. med. Wochenschr., July 31, 1902.
[56] Am. Jour. Med. Sci., 1904, vol. cxxviii, p. 281.
[57] Amer. Medicine, September 20, 1902.

16.67%, and 15.98%, respectively on these 3 days. The eosinophiles vary much; Mastzellen and myelocytes are few. It is not until the eleventh day that the blood picture is that usually considered normal for infants, *i. e.*, with 40% small mononuclears, etc.

This high leucocyte count has been explained as due to abnormal concentration of the blood or to a digestion leucocytosis, but the more rational explanation is that it is the result of the rapid blood formation at that age. Although the blood of normal infants varies much, yet this rather high count may continue until the third or even the sixth year, after which time the blood picture of the adult prevails. During these early years the polymorphonuclear neutrophiles vary from 18 to 40% and the small mononuclears from 40 to 60% of the total number. Often there is a slight increase in the eosinophile cells. Such are the reports of several observers. These figures are not the invariable rule, however, as many a teacher discovers when he attempts to demonstrate a lymphocytosis to a class and uses a new-born baby's blood only to find the picture of a normal adult.

Pathological Leucocytosis.—Leucocytosis of Inflammations and Various Febrile Diseases.—Most of the acute pyogenic infections and many of the acute febrile diseases are accompanied by a leucocytosis, due to an absolute increase of the polymorphonuclear neutrophile cells, which runs roughly parallel to the temperature and which depends for its existence and grade on the activity of the inflammatory process and on the condition of the patient.

The following general statements may be made. Whatever its immediate cause, a leucocytosis represents a reaction of the individual to the disease. In those conditions which usually call forth a leucocytosis a high count means a vigorous reaction, little more, while a low count may mean a poor reaction, hence indicate a poor prognosis, but it also may mean that the infection is so mild that it can elicit little or no reaction.

Diseases differ much in their ability to produce a leucocytosis; or, to put it in a different way, the body defends itself differently against certain diseases; by means of a leucocytosis in some and by a leucopenia in others. In acute lobar pneumonia, scarlet fever and the pyogenic infections there is usually a leucocytosis the degree of which runs roughly parallel to the intensity of the body's reaction, while the absence of a leucocytosis in measles, malaria, and tuberculosis is of great importance in diagnosis. Some diseases may begin with a leucocytosis and end with a leucopenia. This is true of some cases of typhoid fever. Some develop a leucocytosis during the course of the disease, as typhus fever, some cases of influenza, and smallpox. Some diseases, as malaria, ordinarily without a leucocytosis may in severe cases develop one. But in all cases more depends on the organs infected by a given germ than on the germ itself. For instance, typical typhoid infection of the lymphatic apparatus of the bowel causes no leucocytosis but the invasion of the pleural cavity or of the periosteum by

Bacillus typhosis will do so promptly. Tuberculous adenitis causes no rise of the white cell-count but tuberculous pneumonia may cause a high count.

In cases of local infection, as abscess formation, the leucocytosis is a symptom related to the fever and other toxic features and evidently is, like them, caused by, and its severity determined by, the amount of toxine absorbed; for, following an operation which allows free drainage, both quickly drop to normal. For much the same reason the leucocyte count runs quite parallel to the richness of the exudate in pus-cells. In general, the leucocyte count is no indication of the severity of the condition; a simple local felon may cause as high a leucocytosis as an appendix abscess and a fatal pneumonia as little as a small boil.

It is not the formation of an inflammatory exudate alone which governs the leucocyte count, for some patients with free drainage of pus may daily lose enormous numbers of white cells (almost as many as are in the circulation at any one time) (see page 507) and yet show a normal count. This is well seen in some cases of chronic bronchitis, bronchiectasis, cystitis, in various bone and joint abscesses with discharging sinuses, in empyema after operation, etc. The agent causing the leucocytosis seems the same as that causing fever, for they usually run parallel.

One would expect that a great loss of cells in an exudate would cause a diminution of the white cell-count in the blood and there are cases of spreading peritonitis in which this count does for a time fall.

Among the conditions causing leucocytosis are: Acute lobar pneumonia, the best studied example (page 640).

Acute tuberculous pneumonia (page 636).

Acute articular rheumatism (page 644).

Diphtheria (page 634).

Acute cerebro-spinal meningitis caused a leucocytosis in all of 21 cases (Osler); in 4 it was over 40,000; the highest was 47,000. The leucocyte count is of no especial value in distinguishing the various forms of meningitis, since it is present also even in the tuberculous form.

An *acute follicular tonsillitis* usually causes a leucocytosis. This was true of 18 of 26 of our recent cases. (In 12 the count ranged from 10,000 to 15,000; in 3 it was above 20,000; the highest was 27,000.) The temperature was high in all the cases with high counts.

Scarlet fever (page 634).

Mumps. The occurrence of a leucocytosis is disputed.

In *whooping-cough* the leucocytes, especially the lymphocytes, are much increased, the counts averaging 40,000. This leucocytosis is more pronounced the younger the child is. Its early appearance makes it of great value in diagnosis. It begins during the catarrhal stage and, continuing through the paroxysmal stage, reaches its maximum during convalescence. The increase of the count would seem to be due to an increase of the lymphocytes especially but others claim it to be a true leucocytosis.

In Norton and Kohman's case of *anthrax* the leucocyte count was 31,000 per c.mm. of which 81% were polymorphonuclear neutrophiles, 17% small mononuclears and 2% large mononuclears.

Rabies sometimes causes a true leucocytosis of even 25,000, with 98% of the cells polymorphonuclear neutrophiles.

Erysipelas causes a leucocytosis which runs fairly parallel to the temperature. The count ranges between 10,000 and 20,000 in mild and between 20,000 and 30,000 in more severe cases. Its polymorphonuclear neutrophile character is more marked in adults than in children. These cells may reach 92% of the entire count in fatal cases. As the count falls the eosinophiles may rise considerably.

In 6 of our cases the leucocytes were normal in 2, moderately elevated in 2 and were 26,000 and 34,500 in the other 2. The red cells were normal in all.

In *acute ulcerative endocarditis* the leucocyte counts are high as a rule, especially in those cases which run a protracted course. But a leucocytosis is not constant. In some mild cases and in rapidly fatal cases there may be none.

In 6 recent cases at death the counts were 7070, 12,300, 13,600 (it had fallen from 34,000), 17,000, 47,000 and 48,000 (it had risen from 9800).

Acute Poliomyelitis.—In acute poliomyelitis Peabody, Draper and Dochez[58] found a constant and marked leucocytosis. They also found a constant increase in polymorphonuclear cells of from 10 to 15% and a diminution of lymphocytes of from 15 to 20%.

In *intestinal obstruction* the leucocytes rise rapidly, to about 16,000 when the obstruction is partial, and to 20,000 or more when it is complete. If the count reaches over 20,000 cells during the first 24 hours the chances are that gangrene of the bowel has developed. This rise of leucocytes may be of value in a case of suspected post-operative obstruction (Bloodgood).

The *myxedema* which follows a thyroidectomy may be accompanied by a leucocyte count of even 49,000.

Smallpox (page 634).

Cholera.—During the algid stage of cholera the leucocytes may number from 40,000 to 60,000. The count rapidly falls during the stage of reaction.

Pyogenic inflammations, not due to Bacillus tuberculosis, of the serous membranes, the meninges, pleura, pericardium and peritoneum, are accompanied by a leucocytosis which bears some relation to the cellular richness of the exudate but more to the fever. The count varies with the progress of the disease since it may drop to normal while the process is stationary even though the temperature remains elevated and then a slight spreading of the process will cause a rapidly rising count. This is well seen in pelvic inflammations.

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[58] Monograph of The Rockefeller Institute for Med. Research, No. 4, New York, 1912, 97.

In 99 cases of PLEURISY WITH EFFUSION the red cells were practically normal; in 65 of these the leucocyte counts were below 10,000 cells and in but 3 of the remaining 34 cases were they over 15,000. Cabot reports almost exactly the same figures for the Massachusetts General Hospital (314 cases; 33% above 10,000; 6% above 15,000). The low counts are interesting since so many such cases are clearly tuberculous.

In a series of cases all of which were possibly tuberculous the counts were found above 12,000 in 18.9%. In those of this series which were positively tuberculous it was above 12,000 in 9%; in those cases of combined pulmonary tuberculosis and pleurisy with effusion it was above 12,000 in 21.8% while, finally, in those cases of pleurisy with effusion combined with pneumonia it was above 12,000 in 78.5% of the cases.

Empyema.—An inflammatory leucocytosis is the rule in cases of primary pneumococcus empyema and of those cases which follow acute lobar pneumonia. In such cases the leucocytosis is due to an increase of the polymorphonuclear finely granular cells and the white cell count curve will run roughly parallel to that of the temperature. In cases of pneumococcus empyema following pneumonia it is the continuous elevation of the leucocyte count together with the slight fever which leads to the correct diagnosis. This leucocytosis is valuable in excluding serous effusions. In the empyema prevalent during the influenza epidemic of 1917-19, however, a very different state of affairs existed since the count usually was that of the preceding pneumonia even though this was below 3000 cells.

In pneumococcus cases allowed to remain without operation for a long time the count may return to normal.

Fibrinous Pleurisy.—In fibrinous pleurisy a slight secondary anemia is to be expected. The leucocytes in 37 cases which we reported ranged, in 24, from 10,000 to 22,900, while in 13 the counts were normal. Lord found the count above 12,000 in 39% of his cases of fibrinous pleurisy. Since the count was above 12,000 in but 9% of his cases of pleurisy with effusion he suggests that the higher count in fibrinous pleurisy is evidence that this condition is more often a pyogenic infection than is the other.

INFLUENZA is a term which has been applied to a variety of conditions of chest, abdomen or nervous system due to organisms of the streptococcus and pneumococcus groups, to Bacillus influenzæ, etc., which have had this in common, however, either that the condition was epidemic or if sporadic that the infection was particularly prostrating. The epidemic of 1917 to 1919 proved quite satisfactorily that the organism of the disease is as yet unknown, that its infection produces a marked leucopenia and that it prepares the body for secondary invasions by the above-mentioned organisms which explain the various complications which give the more prominent characteristics to the disease, including often a leucocytosis.

If we accept the diagnoses of the past, the leucocytes are normal in about ⅔ of the cases (Cabot) and moderately increased in the rest. Some have stated that in the typhoidal or abdominal form of influenza there is leucopenia.

In almost half of our sporadic cases the count was above 10,000, even 25,000, at the height of the disease. Nearly all the cases in which several counts were made showed early a very low count, from 3,000 to 5,000, even when the temperature was from 100 to 105°, doubtless the count of the influenza itself, then a sharp rise due to a secondary infection.

During the epidemic of influenza of 1918-19 a leucopenia was the rule, the counts averaging about 4,000 cells, while in some cases even in the presence of extensive pneumonia the count was as low as 2,800. In other cases a leucocytosis was a feature of the pneumonia and in 1 case of empyema the count reached 80,000.

The pyogenic processes of mucous membranes which cause fever are usually accompanied by a leucocytosis, as enteritis, urethritis, etc.

In *acute bronchitis* the leucocytosis continues as long as the fever. The counts were between 10,000 and 20,000 in 30 of our 67 cases.

In *chronic bronchitis* the emphysema and attending cyanosis may explain the occasional leucocytosis which was present in just half of our cases. The red cell counts averaged high, the mean being 5,000,000. Of 25 cases in 3 the counts were above 7,000,000 (maximum 7,900,000).

In a case of true *foetid bronchitis*, the leucocyte count was 22,500.

In 11 cases of *bronchiectasis* the leucocyte counts were 20,000 in 2; between 10,000 and 20,000 in 4; and normal in 5 afebrile cases.

Among the *local pus processes* in which the leucocyte count is of value in diagnosis are appendicitis (page 644), pelvic inflammatory disease, abscess of the liver, emphysema of the gall-bladder, ovarian abscess, abscess of the brain, etc.

In *abscess of the lung* counts as high as 60,000 have been reported. In our 3 cases they were 8,100, 12,300 and 12,500. In 2 cases of *gangrene of the lung* they numbered 20,000 and 48,000.

In 25 cases of *gonorrheal arthritis* the mean count of red cells was 4,500,000; the lowest was 3,600,000. The mean leucocyte count was 9 ,000. In 8 of 23 cases the white counts varied between 10,000 and 20,000.

In *perirenal abscess*, 5 cases, the leucocyte counts were between 19,000 and 26,000; *pyelitis*, 4 cases, between 10,600 and 19,500; *pyelonephrosis*, 2 cases, 18,000 and 28,500; *hydronephrosis*, 2 cases, 6,400 and 9,000; and *pyelonephritis*, 1 case, 8,000. In *renal calculus*, 4 cases, the leucocyte counts during the colic were between 12,000 and 18,000.

In *gout* the red cell counts were 5,000,000 or over in all but 2 cases (of 13 cases the lowest was 4,300,000). The leucocyte count rises at the onset of an acute joint attack. (In 18 cases there was a mild leucocytosis from 10,000 to 14,000 in 7 cases.) The variations in the leucocyte count run parallel to the temperature curve and the joint symptoms.

There is a leucocytosis in *diabetic coma* and in *uremia*.

In *dementia præcox*[59] there is a polymorphonuclear neutrophile leucocytosis of 15,000 or less coincident with the onset of the abnormal mental phases for which no satisfactory explanation has been given. In *general paresis* there is often an absolute lymphocytosis, the total number of leucocytes ranging between 7,000 and 10,000, from 35 to 55% of which are small mononuclears.[60]

Following operations there is often a slight *post-operative leucocytosis*, from 10,000 to 20,000, which is not due to infection,[61] which reaches its maximum in the first 12 hours after operation, and which is not accompanied by parallel changes in the temperature or the pulse rate. This leucocytosis continues for not over 36 hours. If, however, the count rises more than 10,000 above what it was before operation and remains elevated for over 2 days one should suspect a post-operative pyogenic complication. The highest count in our series which followed a nephrotomy was 32,000 cells. While the nature of the operation seemed to have little influence on the count, yet the rise did seem to run roughly parallel to the amount of tissue traumatism produced. There is little relation between a post-operative leucocytosis and a post-operative fever due to infection. Chloroform anesthesia, but not ether, can cause a true but transitory leucocytosis.

No sharp line can be drawn between the leucocytosis of infected and of non-infected wound repair except that the latter is on the wane at a time when the former is just beginning.

When the packing of a wound is changed the leucocyte count may rise somewhat. In the case of a closed wound the leucocyte count is a good index of an infection.

The diseases which cause as a rule no leucocytosis are influenza (page 516), typhoid fever (page 639), measles (page 633), and tuberculosis (page 634).

PSEUDOLEUCOCYTOSIS.—Certain blood-changes other than a rise of the white count sometimes occur under conditions which usually cause a leucocytosis and are supposed to have much the same significance. Among these are iodophilia (page 524), degenerations of the leucocytes, fragmentation of their nuclei (as occurs in cancer), the appearance of myelocytes and a relative increase of the polymorphonuclear neutrophiles although the total count does not rise above normal. This is met with in cancer, septicemia, etc.

Leucocytosis of Malignant Disease.—Cases of carcinoma (page 650) and of sarcoma especially (page 652) are frequently accompanied by a leucocytosis of polymorphonuclear cells which disappears after the removal of the tumor. This is frequently true of cancers of the internal organs, especially of the stomach, but not of epithelioma of the skin. This leucocytosis bears little relation to the situation of the tumor unless it metastasizes to the bone-marrow, in which case it may simulate leukemia.

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[59] Barnes, Am. Jour. Insan., April, 1909, vol. lxv.

[60] Cornell, Am. Jour. Insan., July, 1907, vol. lxiv.

[61] Frazier and Halloway, Contrib. from the Wm. Pepper Lab. of Clin. Med., 1902 No. 3. Am. Jour. Med. Sci., September, 1902, vol. cxxiv.

Post-Hemorrhagic Leucocytosis.—Large hemorrhages are often followed by a leucocytosis which begins in from 10 to 15 minutes and which in 1 hour may reach from 16,000 to 18,000. It lasts a few days and then disappears. The increase is of the polymorphonuclear neutrophile cells. Its cause cannot be a new production of cells, since it begins too suddenly. Some attribute this to the tissue lymph which flows into the vessels in order to restore the volume of blood, carrying with it a large number of white cells (but these cells should be small mononuclears), but the nature of the wound is more important, since an injury without hemorrhage can cause a leucocytosis and even a severe hemorrhage resulting from a very slight injury (*e. g.*, that from a gastric ulcer) a slight leucocytosis lasting in some instances but 2 days. Stassano and Billou [62] found that a leucopenia may follow severe hemorrhages and a true leucocytosis smaller ones.

In a case of cirrhosis of the liver with fatal hemorrhage from the stomach the red cell count before death was 1,960,000, hemoglobin 23% and the leucocytes 23,000.

Agonal Leucocytosis.—Since belief in an agonal leucocytosis antedates our knowledge of inflammatory leucocytoses it is more than likely that the leucocytoses of pneumonias were included under this heading. And yet this would not explain all of the high ante-mortem leucocyte counts, *e. g.*, Cabot's case of pernicious anemia which resembled a leukemia. Such cases are rare, it is true. In most diseases the leucocyte count does not change or even drops at death, while the rises which one does find are ascribed by Ehrlich to the slowing of the circulation and hence to the accumulation of the leucocytes along the periphery of the blood-vessels.

Medicinal Leucocytosis.—The administration by mouth or subcutaneously of any one of a very long list of drugs, including the ethereal oils, tonics, myrrh, turpentine, peppermint, etc., may cause a definite leucocytosis. If the drug is administered by mouth the result would seem comparable to a digestion leucocytosis, while if injected subcutaneously the local reaction also may be important. It is of interest that the extracts of certain body tissues also seem positively chemotactic to leucocytes.

The reverse also is true, for blood poisons which destroy the cells, as phenacetin, the chlorates and pyrogallic acid, may cause a drop in the leucocyte count.

Other Causes of a Leucocytosis.—In the case of animals simple violence will cause a leucocytosis. In man, hard work, severe sweating, heat and cold, many vasomotor influences, anything which slows the circulation and causes cyanosis will do the same. Thayer found that in typhoid fever cold baths, especially those which made the patient shiver, would cause a rise of about 6000 cells; the formula remained the same. Violent exercise will cause a leucocytosis; for example, the leucocyte counts of the runners of a 25-mile race rose from 14,000 to 22,000.[63]

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[62] Compt.-rend. Soc. Biol., 55, p. 180.

Mixed Leucocytosis.—By the term mixed leucocytosis was formerly meant an increase of the ameboid and non-ameboid cells; that is, of the granular and non-granular cells. But the discovery that the latter are ameboid robbed this term of all significance. Others used it of a leucocytosis with the presence of neutrophile myelocytes. The best illustration of this is myeloid leukemia in which condition the absolute number of myelocytes may vary from 50,000 to 100,000 per cubic millimeter. In no other condition does the absolute number of myelocytes in the blood rise above 1000 (Ehrlich). In leukemia appear the largest number of eosinophile myelocytes seen, of Mastzellen also, together with all forms of the non-granular cells. The next most important condition in which a mixed leucocytosis may arise is pernicious anemia. In focal infections or malignant nodules of the bone-marrow the blood may appear even leukemic.

And yet almost any leucocytosis could be called "mixed" since the higher the count the younger are the forms which appear in the blood. Especially is this true of children whose blood is very unstable, and particularly of those with diphtheria, anemia, rickets, congenital lues and pneumonia. In the latter condition especially, one may find just after the crisis myelocytes and megaloblasts. Myelocytes have no significance in the blood of adults if they disappear as the count falls, but should they still appear after the count falls their presence might mean exhaustion of the bone-marrow. A mixed leucocytosis is not rare in severe post-hemorrhagic anemias.

Mastzell Leucocytosis.—The only condition in which the Mastzellen of the blood are always increased is splenomyelogenous leukemia. In that disease even 15 or 20% of the leucocytes may be these. Isolated cases with an increase of these cells in the blood have been reported, as of cancer, septic bone disease, various skin diseases and even chlorosis. The difficulty of recognizing these cells in specimens stained with methylene blue mixtures should be emphasized.

Leucocytosis with an Increase in Endothelial Leucocytes.—The endothelial leucocytes are increased absolutely in typhoid fever, measles, and especially in malaria in which disease even 20 to 30% of an almost normal count may be these cells, a point of great value in diagnosis.

Lymphocytosis.—Ehrlich classified leucocytoses as active, passive and mixed. The ordinary leucocytosis is "active" because it is the actively ameboid cells which are increased. These are supposed to have entered the circulation in abnormal numbers in response to a positive chemotactic agent. Ehrlich called a lymphocytosis "passive" because he supposed these cells to be passively and mechanically washed out of the lymphatic tissue by the circulation.

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[63] Larrabee, Jour. of Med. Research, 1902, vol. ii.

The lymphocytes are ameboid on a rather hot stage (44° to 46°C.). They migrate into the tissues in certain skin diseases, also in tuberculosis, lues and malignant disease. They are the cells of the tuberculous pleural exudates. Under these conditions they certainly are ameboid.

Rous [64] found that in man a lymphocytosis may follow active exercise unless this is very severe (as a 25-mile run) in which case there may be a decrease in the absolute number of mononuclear cells of the blood. This increase is not due to an increased flow of lymph since the increase of these cells in the blood is even twice that in the thoracic duct and is a greater addition than any simple lymphagogue, as glucose, can produce.

These cells contain a lipase capable of splitting wax and fat to glycerine and fatty acid, which is interesting since the bodies of tubercle bacilli contain about 30% of waxy substances.

Rous found that normally the lymph of the thoracic duct furnishes the blood with a large proportion of its lymphocytes and that under normal conditions this supply is quite constant.

Physiologically, a lymphocytosis exists in infants and during a digestive leucocytosis. The mononuclear cells are definitely increased at high altitudes, averaging 43.6% (polymorphonuclear neutrophiles 54%).[65] Pathologically it occurs in the simple gastro-intestinal disturbances of children (page 647), in whooping-cough (page 513), in cervical adenitis, during the reaction to tuberculin, in malignant lymphomata and in sarcoma multiplex cutis. The greatest increase is in lymphatic leukemia in which disease over 90% of the 140,000 or more cells may be mononuclears. They are absolutely increased in splenomyelogenous leukemia, while after a splenectomy they may slowly increase to even twice their normal number, which increase begins about a month after the operation and continues for even 1 year.

The best illustrations of the chronic diseases with a lymphocytosis are hereditary lues and severe rickets. The statement is often made that a lymphocytosis is common in chlorosis, pernicious anemia, debility, late typhoid fever, Graves' disease, hemophilia, scurvy and during thyroid treatment, but as a rule the increase is only relative.

THE LEUCOCYTOSIS OF CHILDREN is often chiefly a lymphocytosis, *e. g.*, the case with enlarged tonsils mentioned by Churchill, with a count of 20,000, 70% of which were small mononuclears. In the diagnosis of lymphatic leukemia these cases should be remembered. The total leucocyte count may be low and yet a true lymphocytosis exist, as in a recent case of amebic dysentery with a count of 2,500 cells 68% of which were small mononuclears.

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[64] Jour. of Exp. Med. 1908, vol. x; Proc. of the Soc. for Exp. Biol. and Med. 1907, vol. iv.

[65] Webb and Williams, Trans. of the Fifth Annual Meeting of the National Assoc. for the Study and Prevention of Tuberc., p. 231.

Leucopenia, or a count below 5000 cells per c.mm., may result from the reduction in 1 group of cells or from a general reduction of all groups. The former is seen during typhoid fever. A leucopenia is said to be the first stage of a leucocytosis. In tuberculosis of lymph-glands the count may be even below 600 cells. In aplastic anemias it may fall as low.

Ehrlich mentioned an interesting case of general lymphosarcoma with but 0.6% of small mononuclears in the blood.

Conditions with leucopenia have been reported under the name "alymphæmic lymphomatosis." Such a case was reported by Schwartz with acutely swollen glands and fever whose leucocyte count was only 600 per c.mm. and all of them lymphocytes (no autopsy).

The relationship between leucopenia and infection of the abdominal organs deserves mention. Infections with Bacillus typhosus usually produce a leucopenia when the local infection is chiefly abdominal, but they produce a leucocytosis when other organs as pleura, lung, etc., are involved; tuberculous peritonitis and "abdominal influenza" cause no leucocytosis.

The leucocyte count may fall to a low figure during the convalescence of some fevers. At the end of typhoid it is often as low as 2000 cells.

In a recent case of hemoglobinuria the red cell count was 2,500,000 and the leucocyte 950 per c.mm.

Cases of starvation or malnutrition from any cause have a low leucocyte count; for example, voluntary starvation (page 597) and that due to disease as cancer of the esophagus (page 651). In one of our cases of ulcerative colitis the patient's red count was 2,100,000, and the leucocytes 700 per c.mm. In influenza even with pneumonia the count may drop to 2800 or lower. In measles there is often leucopenia.

In 1 of our cases of measles, No. 9621, a man 22 years old, the count was 3600 per c.mm. at the height of the disease.

In acute miliary tuberculosis (page 637) the counts are often very low. In all chronic intoxications as alcohol, morphia, lead, ether, mercury, arsenic (hence the drop in leukemia?), leucopenia is the rule. Benzol will produce a marked leucopenia.

Eosinophilia.—By eosinophilia is meant an absolute increase of the eosinophile cells in the circulating blood. The normal percentage of these cells varies from 2 to 4% and many use this term of conditions in which the percentage is increased; but the term should be limited to those cases in which the absolute number of these cells is above 250 per c.mm.

Those conditions in which these cells are increased are so varied that they have been well termed the most capricious cells of the blood.

I. There is a PHYSIOLOGICAL EOSINOPHILIA during childhood.

II. DISEASES OF THE HEMATOPOIETIC ORGANS. 1. *Bone-marrow*—Diseases of the bone-marrow often produce an eosinophilia. (*a*) In splenomyelogenous leukemia there is usually but not always an absolute increase of these cells, even 29,000 per c.mm. (Zappert). In a few cases they have been reported absent. In (*b*) sarcoma of the bone-marrow, page 652, (*c*) osteomyelitis and (*d*) osteomalacia they are sometimes present in great numbers.

2. *Spleen.*—About 1 year after the removal of the spleen there slowly develops an eosinophilia which lasts for several months. These cells are increased to from 30 to 50 times their normal number and may make up even 36.6% of the total leucocyte count. A somewhat similar condition is present in cases with chronically enlarged spleen, in which the percentage of these leucocytes may vary from 7 to 12, and in new growths of the spleen. The reason may be that these spleens are functionless.

3. *Lymph-glands.*—That an eosinophilia may result from disease of the lymph-glands is not certain. In support of such conditions have been cited cases with extensive metastases of carcinoma to these glands and eosinophilia but metastases to bones also were not excluded. In one case with such bone metastases the eosinophile cells numbered 60,000.

III. Asthma.—During the paroxysms of true bronchial asthma from 10 to 20% and in a case of Billings between 53 and 54% of the leucocytes may be eosinophile cells. This has considerable value in the differential diagnosis between true asthma and asthmatic attacks due to other causes. In emphysema these cells are also increased, in 1 case numbering 53.6% of a total of 8300 leucocytes.

IV. Skin Diseases.—Many skin diseases produce an eosinophilia, which seems to depend more upon the extent of the lesion than on its nature. The cells in the pustules of some conditions are sometimes all eosinophiles.

In a case of pemphigus reported by Zappert there were 4800 eosinophiles per c.mm. of blood; in 1 of pemphigus vegetans of the Baltimore clinic the total count was 20,400 leucocytes, 2.6% of which were eosinophiles, and on another day 11.6%; in pellagra and psoriasis these cells are sometimes increased; in urticaria even 60% of the total count may be these cells; in a case of the Baltimore clinic of purpura with cyanosis 11% of 52,000 leucocytes and later 25% of a count almost as high were eosinophiles; in certain cases of eczema they are increased; of 2 cases of scleroderma in this clinic, in 1 they constituted 2.4% of 7000 and in the other 3.3% of 10,500 leucocytes; in 5 cases of purpura simplex the red cells and hemoglobin were practically normal but 3 had a leucocytosis of from 10,100 to 40,600 of which from 11.3 to 25.6% were eosinophiles. (In 1 of these three cases, one with myositis the eosinophiles were 12.1% of 40,600 cells; in another 25.6% of 20,300.) In 1 of 3 cases of purpura rheumatica there was a leucocytosis of 13,300; in 1 of 3 of Henoch's purpura they were 10,000. Of 2 cases of purpura hemorrhagica in 1 the eosinophiles numbered 4.5% of 6600, in another 9.4% of 5200. In 1 case of morbus erronum 18% of the 5000 leucocytes were eosinophiles. Later they were normal. Chemical irritation of the skin, for instance by mercuric chloride, may increase these cells even to 14%.

V. **Parasites.**—Any animal parasite, from the harmless pin-worm to the most malignant uncinaria, may cause an eosinophilia, but it does not always, nor does its degree bear any relation to the severity of the infection.

In *amebic dysentery* of children especially there may be a slight eosinophilia.

In 1 of our cases of amebic dysentery, No. 8174, a man 36 years old, these cells were 8% of a total count of 8,800.

Brown demonstrated this as a most important point in the diagnosis of *trichiniasis*. In 1 of his cases 68.2% of the 35,000 leucocytes were eosinophiles. This eosinophilia is not always present in trichinosis as in one case reported by Howard and another by Da Costa, but these cases are rare. In Brown's case they fell gradually to normal. The neutrophile cells in these cases are relatively and absolutely low. Brown was unable to obtain any Charcot-Leyden crystals from the blood and so suggests that some other element than an increase of eosinophile cells is necessary for their presence.

In *uncinariasis* the percentage of eosinophile cells usually ranges from 8.2 to 10 and in 1 case reached 72% of the count. Our highest count was 13% of a total of 7400 leucocytes. In 1 case infected with *Taenia saginata* the eosinophile cells were 34% of the total count; of *Ascaris lumbricoides*, 19%; *Oxyuris*, 16%; *Strongyloides intestinalis*, 13.5%; *Bilharzia*, 20%. In *filariasis* they vary from 4 to 17% but in Calvert's case they reached 22% during the day (in others the maximum is at night). Calvert thinks that the grade of the eosinophilia depends on the acuteness of the attack and so it may be absent in long-standing cases. He found that the number of these cells in the circulation bears an inverse relation to the number of embryos, the former increasing during the day when the embryos disappear. In the diagnosis of *hydatid cysts of the liver* an eosinophilia is considered important. It varies from 7 to 20% and in 1 case reached 40%. During the afebrile stage of malaria these cells have risen to 20.4%. In *dracontiasis* they are reported as from 6.4 to 36.6%.

VI. **A post-febrile eosinophilia** may develop after many fevers but in most cases the increase is only to the upper limits of normal. In *scarlet fever* these cells may vary from 8 to 15% during the course of the fever but in all other fevers they first diminish and then rise as the temperature drops. After the crisis in *pneumonia* they may number 430 (5.7%); in acute articular rheumatism, 970 (9.4%); in malaria 1 day after the chill, 1486 (20.34%); in *varicella* they may rise to 16%; in measles to 5%; and in rickets to 20%.

VII. During a **positive tuberculin reaction** these cells may fall and then rise even to 3220 (26.9%). In 1 case reported by Grawitz their absolute number was 41,000 of a total of 45,000 leucocytes.

VIII. These cells are often increased in **diseases of the genital organs;**

in all ovarian diseases except cancer, in 10 of 18 cases with ovarian cysts and abscesses and in gonorrhea, especially posterior urethritis and prostatitis.

IX. In **malignant disease** the eosinophiles sometimes make up from 7 to 10% of the leucocytes while in one case of lymphosarcoma they numbered 60,000 cells.

X. After certain **medicines,** as camphor (in which case they may rise to 9%) or the inhalation of carbon dioxide, the eosinophiles are sometimes, though rarely, increased.

XI. In diseases of the **sympathetic nervous system.**

In the diagnosis of animal parasites, of asthma and of diseases of the bone-marrow the count of the eosinophile cells may be of great value. These cells certainly appear in the blood and tissues in response to some specific positive chemotactic agent. The best recent review of this subject is that of Howard.[66] We find in some pus, some sputa, etc., practically only eosinophiles, although the differential count of the circulating blood of these patients may be normal.

It is important that in the blood of some rare cases we find a group of leucocytes which might be either neutrophiles or eosinophiles (see page 499). Were this the testimony of but few their technic or their judgment might be suspected, but several have reported such cases.

Iodophilia.— By iodophilia is meant the presence in the blood of leucocytes whose protoplasm either takes a brownish red color or contains granules which take that color when the specimen is treated with iodine (the "intracellular reaction") or the presence in the plasma of similarly stained granules from 2 to 8μ in size (the "extracellular reaction").

If normal blood is thus treated all the blood elements will take a bright yellow stain.

The reagent used contains: iodine, 1 gm.; potassium iodide, 3 gms.; water, 100 cc.; and gum arabic, 5 gms. A drop of this solution is placed on a slide and an unfixed smear of fresh blood is pressed down into the drop. The excess of stain is removed with a piece of filter paper applied at the edge of the cover-glass while this is held firmly against the slide. In judging the degree of iodophilia both the number of the cells and the degree to which these are affected are considered. Since all leucocytes contain some glycogen one must assume in iodophilia a special degeneration of those leucocytes which give the reaction, perhaps of toxic origin. which increases their affinity for iodine.

This reaction is positive in a great variety of conditions. Locke considers the test independent of but of nearly equal value with a leucocytosis. It is met with in all the anemias except chlorosis, in leukemia, in nearly all cases of septicemia, especially those with leucocytosis, in cases with purulent exudates and especially in pneumonia. It is invariably present in severe

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[66] The Jour. of Med. Research, Dec., 1907.

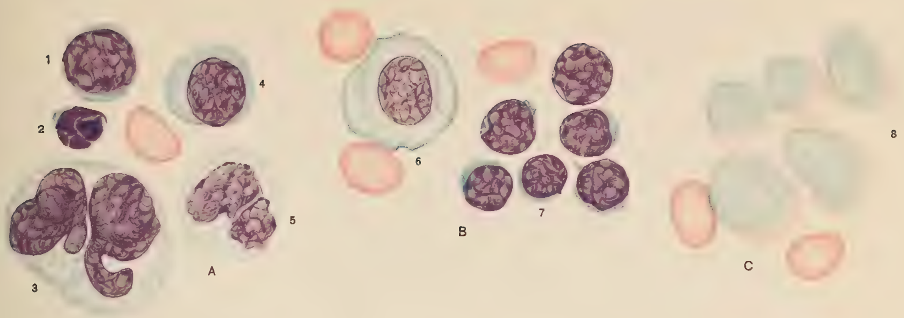
PLATE II

A, B, and C are groups of cells from three cases of Acute Lymphatic Leukemia.
  A. Cells from a case of the large-celled variety.
  B. Cells from a case of the small-celled variety.
  C. In this case the cells were almost achromatophilic, with the protoplasm slightly acidophilic.

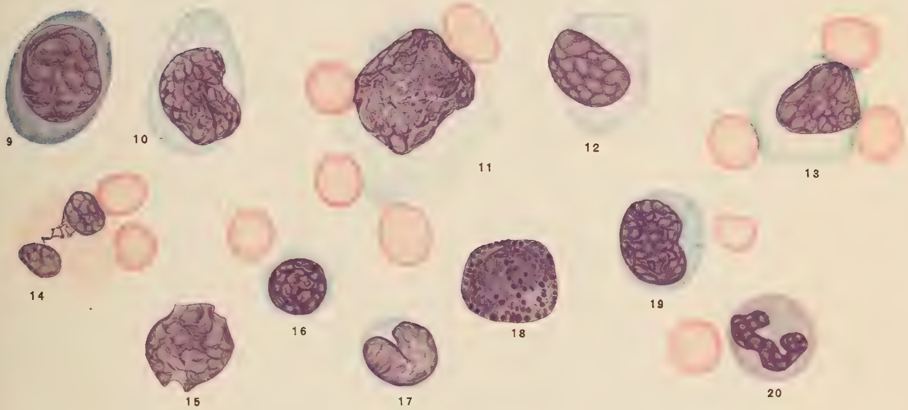
Leucocytes from Normal Blood and Malaria (in which condition is a large number of large mononuclear nongranular forms).

9, 10, 13, 19. Large mononuclear cells.
11. A giant mononuclear cell.
12. A mononuclear cell with the Wolff-Pappenheim granules.
14. An eosinophile leucocyte.
15. A naked nucleus.
16, 17. Small mononuclear cells.
18. Mastzell.
20. A neutrophile leucocyte.
21. Trypanosoma gambiense.
22, 24, 25. Red cells with Grawitz's basophile granulation.
23. A blood platelet.

PLATE II


1
4
2
6
8
7
5
3
A
B
C

LYMPHATIC LEUKÆMIA.


9
10
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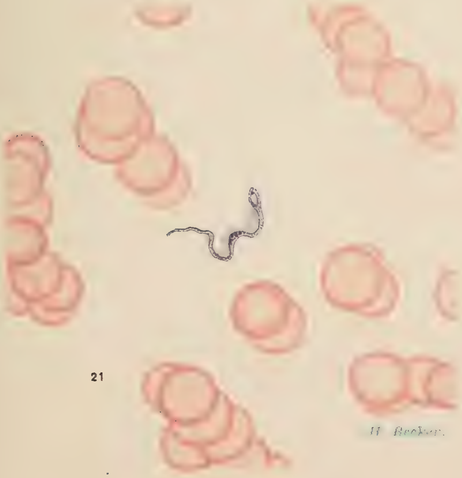
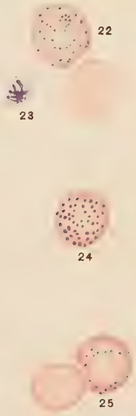
LEUCOCYTES OF NORMAL BLOOD AND MALARIA.





TRYPANOSOMA GAMBIENSE,
FROM A CASE OF "SLEEPING SICKNESS."
STAINED WITH HASTING'S MODIFICATION OF
ROMANOWSKI'S STAIN. ALL DRAWN TO SAME SCALE.

BASOPHILE GRANULES
OF RED BLOOD CELLS.
F. S. Lockwood.

septic conditions [67] and is valuable in this diagnosis if there is no leucocytosis. Another claim made is that this test is negative in cases of ovarian cyst with twisted pedicle and in other conditions without pus formation, even though there is a high leucocytosis.

BLOOD PLATELETS

Blood Platelets, Blutplätchen, Plaques (Kemp, Osler), Hematoblasts (Hayem), (Plate II, 23). In the blood are the so-called "third corpuscles," small bluish bodies without nucleus or cell membrane and containing no hemoglobin, about 3μ in diameter, round, oval, or rod-shaped, according to the view-point and not biconcave. In an ordinary fresh blood preparation they have a peculiar bluish refractivity like the protoplasm of a non-granular leucocyte, but they stain like nuclear material. Platelets when perfectly fresh are slightly granular, but when removed from the blood-vessel they at once become hyaline, glassy and very sticky, then pale and disappear or they unite in masses of 2 to hundreds and disintegrate rapidly, even in a few seconds forming the so-called Schultze's "granular masses" (see Fig. 125), from the periphery of which radiate fibrin strands and at the edges of which are vacuole-like areas, the so-called "viscous metamorphosis" of Eberth or the "mucoid degeneration" of Osler.

In normal blood specimens stained with the usual Romanowski mixtures, they are seen in groups of from 1 to 10. They would seem to consist of protoplasm, sometimes hardly visible and sometimes definitely stained and swollen almost to the size of a red corpuscle [68], and of nuclear material in rows of blue or reddish dots sometimes arranged in a spherical mass.

When the platelets rest on cells they may closely resemble malarial parasites.

Their size in the fresh specimens varies from 2.5 to 5μ in diameter (Determann); 1.5 to 3.2μ (Osler); 2 to 7μ (Preisich and Heim). In general their size varies inversely as their number; that is, the more the platelets the smaller they are. Their fragility also is more marked when they are increased. It is best to study them at a temperature not over 40°F., for then their changes are much slower, requiring minutes instead of seconds. Some soon show clear areas, either in the center or on one side, or on the whole periphery; others become crescents, triangles, quadrangles, spindles, threads, etc. (see Fig. 125, *a*, *b*).

It is customary to call anything in the plasma a platelet which is smaller than a red blood-cell and which does not contain hemoglobin. Buds from red cells lose their hemoglobin, become granular or glassy "and cannot then be told from platelets;" "inner bodies" extruded from red cells, after undergoing certain degenerative changes, "cannot be told from (degenerated) platelets." This is a mistake. The term "platelets" should be reserved for bodies which answer the above description especially as regards their peculiar bluish refractibility, their stickiness and their tendency rapidly to disintegrate. Anything floating in the plasma of the blood specimen for more than a few seconds is not a platelet no matter how much it may resemble it, unless some special fixing fluid had been used.

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[67] Locke, Jour. Med. Research, Jan., 1902.

[68] See also Puchberger, Virchow's Arch., 1903, vol. clxxi, p. 181.

Specimens of the platelets are best obtained by placing on the well cleaned tip of the finger a drop of suitable reagent, pricking the skin through the drop so that the blood will mix with this fluid before coming in contact with the air, and making a fresh preparation of this mixture. The reagents used are the following: Picini's fluid (mercuric bichloride 2; sodium chloride 4; glycerin 26; and distilled water 226); Hayem's fluid (water 200 c.c.; sodium chloride 1 gm.; sodium sulphate 5 gms.; potassium iodide solution [water 100; potassium iodide 5; iodine in excess] 35 c.c.); Kemp's fluid (0.9% sodium chloride solution in 2.5% formalin); Determann's fluid (distilled water 160; glycerin 30; sodium chloride 1; sodium sulphate 8; methyl violet 0.025 parts); or, a 10% sodium metaphosphate solution.

To count the platelets some estimate in a fresh specimen prepared as above the relative number of platelets and red blood-cells and then make a count of the red cells. From this data the number of platelets per cubic millimeter may be easily calculated. Helber [69] counted them directly, using a leucocyte pipette (which gave a dilution of 1:30), 10% sodium metaphosphate as the diluting fluid and a ruled slide similar to the ordinary counting-chamber, save that the thickness of the blood-film is 0.02 mm.

The normal number of platelets per cubic millimeter has been found 250,000 (Osler); 225,000 (Determann); 245,000 (Enden); and from 190,000 to 260,000 (average 228,000) (Helber). The count varies in the same person at different hours of the day so that a change in their number must be considerable to be clinically important. In the new-born for the first few days the count is very low.

It is hard to classify diseases on the basis of the platelet count but most agree that they are increased in all the secondary anemias but especially the post-hemorrhagic and that during blood regeneration they may bear a relation to the red blood-cells of 1:10. They are increased in chlorosis, but are decreased in pernicious anemia and in any severe anemia which is doing poorly. Their greatest increase is in spenomyelogenous leukemia while in lymphatic leukemia their count is very low (Pratt). They are increased in chronic diseases with cachexia and in conditions with general malnutrition.

During acute fevers of long duration they first diminish but during the third or fourth week as the patient begins to get weak they may increase. In typhoid fever a rapid diminution is considered a bad sign (Turk). In short, sharp fevers there is at first a decrease, then an increase, the curve often resembling that of the leucocytes. The more acute, the more severe, the more threatening the disease and the higher the temperature, the fewer the platelets, so that in severe malaria and pneumonia not one may be found. After the temperature drops, especially if by crisis, the platelets may rise above normal in 24 hours and continue so for 2 to 3 days, then return to normal. In erysipelas and septicemia there is no preliminary decrease but an increase from the start. In acute articular rheumatism there is a great increase in these corpuscles. Four cases have been reported in which the platelets were totally absent: a moribund case of pneumonia, one of nephritis, a case of pernicious anemia and one of purpura.

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[69] Deutsch. Arch. f. klin. Med., 1904, Bd. 81, p. 316.

Pratt [70] found no relation to exist between the coagulation time of the blood and platelet count. The platelets have been shown by Wright [71] to arise from the peripheral portion of the protoplasm of the megakaryocytes or bone-marrow giant cells by a pinching-off process.

Previously their origin was one of the most disputed points in hematology. Schultze and Howell considered them fragments of broken down leucocytes; Bizzozero said they were independent corpuscles, a view which Osler also held; Löwit said, artefacts, while Hayem considered them very young red blood-cells. Then for several years it was agreed that they were independent corpuscles until in 1897 Arnold taught that they were fragments constricted from red blood-cells or fragments of cells which had gone to pieces. Maximow [72] considered them the extruded inner body of the nucleoid of red cells. Engel thought them remnants of the nucleus. Preisich is one of the last to insist that they are the extruded nuclei of the red blood-cells, hence are in constant process of formation; that animals with nucleated reds have no platelets; that platelets increase as the reds increase, and (this point is hardest to accept) that eosinophile leucocytes are phagocytes which have filled themselves with platelets.

The next work of importance is that of Deetjen[73] who, using a special agar plate containing sodium metaphosphate, considers that he has proved them independent cells, motile and nucleated.

Wlassow was an especially severe critic of Deetjen's work. He pointed out that although the platelets thus treated do change their shape, yet they never show true ameboid motion. He, therefore, still believed that they originate in the red cells. Wlassow's argument is interesting. He made quickly a fresh specimen of blood, then ran under the cover-glass a drop of ⅕ concentrated (!) mercuric chloride solution; at once the red cells become granular and a small refractive hyaline area appears, usually at the periphery of the cell. From this a bud develops which is sometimes an irregular mass of granules. This increases in size, may become thorny, and then separates from the corpuscle as a platelet. These bodies may or may not contain hemoglobin. Those which do, later lose their color.

Kemp, as a result of his recent interesting work on the blood at a high altitude, is confident that some platelets do contain hemoglobin, and hence is in doubt as to their origin.

Then came the important work of Wright, who by histological methods found an independent origin for them in the bone-marrow, and that of Cole[74] who showed that a serum which will agglutinate blood platelets will not agglutinate red corpuscles. This certainly is strong evidence against any genetic relation between platelets and red blood-cells.

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[70] Jour. of Med. Research, Aug., 1903.
[71] Arch. f. Path. Anat., 1906, vol. 182, p. 55.
[72] Arch. f. Anatomie u. Physiologie, Anat. Abth., 1899.
[73] Virch. Arch., May, 1901, vol. clxii.

CHEMISTRY OF THE BLOOD

Hemoglobinemia is a condition of the blood characterized by the presence of free hemoglobin in the plasma. This may be demonstrated by examining the spectrum of the plasma obtained by centrifugalizing the fresh blood, or that of the serum after the corpuscles have separated by clotting. Hemoglobin set free in the plasma is transformed as rapidly as possible in the liver to bile pigment causing hypercholia and often a definite jaundice, but if the destruction of red cells involves at least a sixth of their total number the liver is not equal to the task and hemoglobinemia results.

Hemoglobinemia may follow a wholesale destruction of red blood-cells. This may, as happens in severe skin burns, take the form of fragmentation of the corpuscles, in which case some of the fragments may be picked up by the spleen (causing acute "spodogenic splenic tumor") and other internal organs while the hemoglobin of other cells becomes free in the plasma and, if in sufficient amount, causes hemoglobinemia. In other cases certain blood poisons lake a great many red corpuscles in the blood stream. Among these are the toxins of the acute infectious fevers, mentioned on page 601, and the malarial parasite. As cause of the very interesting PAROXYSMAL HEMOGLOBINURIA one assumes an antecedent hemoglobinemia. This, however, can seldom be proved although a lowered resistance of the red cells to mechanical injury has been demonstrated. The best explanation of this condition would seem to be the following: While in the blood of 25% of all persons there is an isohemolysin capable of dissolving the red blood-cells, not of their own blood or of other individuals belonging to the same blood group with themselves (see page 588), but of certain individuals of other groups, patients subject to attacks of paroxysmal hemoglobinuria have in their plasma a hemolysin of amboceptor-complement nature which differs from other isohemolysins in that it is capable of dissolving the corpuscles of these patients' own blood (as well as those of other individuals) and which needs for its activation the sudden change from a low to a high temperature (Moss).

Methemoglobinemia is the condition characterized by the presence of methemoglobin in the circulating blood. This may be the result of the action of certain poisons, such as potassium chlorate, antifebrin, acetanilid, etc. In these cases the pigment is found both free in the plasma and intracellular. In certain "idiopathic" cases associated with weakness, cyanosis and diarrhea, the methemoglobin may be entirely intracellular. The presence of this pigment may be demonstrated by the spectrum of the blood after this has been diluted sufficiently with water. It consists of the 2 bands of oxyhemoglobin and also 1 in the red which extends from λ620 to λ645 (shading off on the 2 sides to λ615 to λ650) and which promptly disappears on the addition of ammonium sulphide.

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[74] Johns Hopkins Hosp. Bull., 1907, p. 261.

The Blood in Carbon Monoxide Poisoning.—The diagnosis of severe cases of carbon monoxide poisoning is sometimes suggested by the macroscopic appearance of the venous blood which has a slightly brighter red tint than normal, but the condition is proven by the spectrum of a few drops of the blood sufficiently diluted with water. The 2 bands of the spectrum of carbon monoxide hemoglobin resemble closely those of oxyhemoglobin, except that they are slightly nearer the violet end of the spectrum and do not unite to form the single band of reduced hemoglobin on the addition of a little ammonium sulphide. Sahli warns us not to place too high an estimate on the test. Some men are so susceptible to this gas that they show severe symptoms of poisoning before carbon monoxide hemoglobin can be demonstrated while in other cases this test will soon cease to be positive after the patient has breathed fresh air although the toxic symptoms may continue for some time later.

Sulph-hemoglobinemia, first distinguished from methemoglobinemia by van der Bergh, has attracted considerable attention since the paper of West and Clark [75] appeared. This condition is often associated with cyanosis, headache, great weakness and obstinate constipation. All sulph-hemoglobin is contained in the red cells, none is free in the plasma. No free H_2S can be demonstrated in the blood of these patients.

The blood spectrum of these cases shows the 2 bands of oxyhemoglobin and a third similar in position to that of the methemoglobin, but not quite so far in the red (it extends from λ 610 to λ625) and does not disappear but rather is intensified by the addition of ammonium sulphide.

Bilirubin and Urobilin in the Blood.—For the detection of bilirubin and urobilin in the blood, Conner and Roper [76] suggest the following modification of Syllaba's Method.

Five cubic centimeters of clear blood serum are diluted with 10 c.c. of distilled water, 0.5 gm. of powdered sodium sulphate added, then 1 c.c. of 5% acetic acid; then it is coagulated by heating it briefly on a water-bath. It is then filtered. The color of the filtrate, usually almost colorless or faintly pink, does not always indicate the presence or the absence of urobilin. It is necessary carefully to examine all filtrates spectroscopically after the addition of 2 or 3 drops of Lugol's solution, using a large spectroscope and absorption cells from 1 to 4 cm. in depth. (A small pocket spectroscope will give fairly satisfactory results if the filtrates are clear.) For confirmation, the filtrate is first neutralized and then tested for the green fluorescence with Schlesinger's zinc-acetate solution. The specimen is allowed to stand 24 hours before it is pronounced negative. The precipitate obtained as above, usually very faintly yellow or white in color, is boiled for a few minutes on a water-bath with from 20 to 30 c.c. of 5% acid alcohol (hydrochloric acid 5 parts and 95% alcohol 95 parts) and filtered. This filtrate is sometimes colorless, sometimes green and sometimes yellowish pink. The colorless filtrates contain neither urobilin nor bilirubin; the green filtrates contain bilirubin and on spectroscopic examination occasionally show a band of urobilin; the pink filtrates contain only urobilin.

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[75] Medico-Chirurgical Transactions, vol. xc, 1907.

[76] Arch. of Int. Med., 1908, vol. ii, p. 532.

The Quantitative Determination of Bilirubin in the Blood.—Conner and Roper recommend for the quantitative determination of bilirubin in the blood a slight modification of Gilbert's method; that is, the blood serum is diluted until Gmelin's test is no longer positive. This reaction is supposed to disappear when the solution of bilirubin reaches approximately 1 : 40,000 and the assumption is made that albumin, hemoglobin, indican and lutein do not interfere with the play of colors. In fluids rich in albumin, however, the characteristic play of colors of Gmelin's reaction is seen only when the bilirubin is present in relatively strong concentrations; for example, between 1 : 3,000 and 1 : 5,000. In dilutions of from 1 : 7,000 to 1 : 11,000 one gets at the point of contact a distinct bluish-green ring, and in weaker solutions the blue ring becomes finer and has a violet rather than a green reflection, but nevertheless remains distinct up to a dilution of about 1 : 40,000.

A series of dilutions of the blood serum to be tested is made, using 8 or 10 flat-bottomed, cylindrical glass tubes of standard size (that is, they are 4 or 5 cm. long with an inside diameter of exactly 1 cm. It is convenient to have a block of wood or a frame in which these tubes can be set in a row.) Three pipettes are necessary: One holding 1.5 c.c. and graduated accurately to 1/20 c.c. is used for measuring the blood serum; one holding 2 c.c. and graduated to 1/4 c.c., for measuring the diluting fluid ("artificial serum"); and 1 with a tapering point, for measuring approximately 1/4 c.c. of the nitric acid reagent. Two reagents are required. The first, the artificial serum, is made by mixing well the whites of several eggs with an equal volume of 0.7% sodium-chloride solution and placing this on ice for 24 hours. The supernatant liquid is then decanted and to it is added caustic soda in the proportion of 0.3 gm. to 100 c.c. It is then allowed to stand 3 or 4 days, during which time a precipitate forms which will carry down the coloring matters of the egg-white leaving a perfectly colorless liquid which in its fluidity, albumin content and alkalinity closely resembles blood serum. This should be kept cool. It should be freshly made at least each month. The nitric-acid reagent consists of 200 c.c. of pure HNO_3, 100 c.c. of distilled water and 0.06 gm. of sodium nitrate.

The necessary amount of blood serum for a test may be obtained from 15 to 20 c.c. of blood obtained by venepuncture and allowed to clot.

Into each of 6 of the glass tubes is measured with the second pipette exactly 0.5 c.c. of artificial serum and then, using the first pipette, increasing amounts of blood serum (*e.g.*, 1/20 c.c. in the first tube, 2/20 in the second tube, 3/20 in the third, 5/20 in the fourth, 8/20 in the fifth, and 12/20 in the sixth tube). The contents of each tube are mixed by shaking and then underlaid with 1/4 c.c. of the acid reagent. Or, the artificial serum and the blood serum may be mixed in a beaker and then superimposed in the proper tube over the nitric acid. This latter method gives a sharper end-reaction.

After the tubes have stood for exactly 30 minutes they are examined against a white background in good daylight (but not in direct sunlight). The examiner's back should be to the light and he should hold the tubes somewhat above or below the level of his eyes. That tube in which the dilution of blood serum is weakest in which a faint but distinct blue ring can be seen, is assumed to contain a solution of 1 : 40,000 bilirubin.

The strength of bilirubin in the initial blood serum can then be readily calculated by the following formula:

$x\ \frac{10+a}{40{,}000a}$, in which a = the number of twentieths of cubic centimeters of serum used.

Example. If the tube with the end reaction contains $\frac{5}{20}$ c.c. of blood serum, then

$$x = \frac{10+5}{5} \times \frac{1}{40{,}000} = \frac{3}{40{,}000} = \frac{1}{13{,}333}$$

The original blood serum, therefore, contained 1 to 13,300 of bilirubin.

The series of tubes should be so prepared that in at least 1 tube no blue line can be seen. It is important that the tubes be examined just half an hour after they have been prepared, since the reaction tends to become stronger on standing and tubes which contain no blue line in 30 minutes may after an hour or two.

Since the blue ring can be obtained with the undiluted serum of some normal persons Gilbert and Hirscher believe that normal human blood, like that of certain of the lower animals, contains a minute quantity of bilirubin. They found this physiologic cholemia to correspond to a bilirubin strength of between 1 : 28,000 and 1 : 40,000, an average of 1 : 36,500.

By this test slight grades of and fluctuations in jaundice may be detected and measured. It has been of assistance in distinguishing between appendicitis and cholecystitis.

REACTION OF THE BLOOD

To litmus the blood is alkaline. At least 35 methods have been proposed to determine the amount of this alkalinity but with little success since these methods are too inaccurate; and even though they were accurate the titratable alkalinity they would measure would have little value in medicine.[77] By alkalinity the physical chemist means a preponderance of free OH-ions in the blood. According to this standard the blood is slightly alkaline when arterial and quite neutral when defibrinated.

Several forms of alkalinity must be considered and that of physical chemistry must receive first attention.

Pure water, the standard of neutrality, at 20°C. contains approximately 1/10,000,000 grams of H-ions to the liter and an equivalent number of OH-ions. That is to say, pure water is a 1/10,000,000 normal acid and a 1/10,000,000 normal alkali. This as expressed in logarithmic notation is 10^{-7}N, but written for the sake of simplicity, pH^7. If we have less than 1/10,000,000 gram of hydrogen-ions in one liter the solution is less acid than water, that is, it is alkaline. That is, pH^8 means actually 1/10,000,000 N alkaline. The higher the exponent, the more alkaline, that is, the less acid, the solution.

pH^1 = N/10 acid
pH^6 = N/1,000,000 acid
pH^7 = Neutrality
pH^8 = N/1,000,000 alkali
pH^{14} = N/10 alkali

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[77] Strouse, Johns Hopkins Hospital Bulletin, Vol. 19, p. 139, May, 1908. [77a] In the preparation of the following sections on the chemistry of the blood we have used freely Gradwohl and Blaivas' "Newer Methods of Blood and Urine Chemistry".

The reaction of the blood serum always lies between pH^7 and pH^8. The neutral point, pH^7, is reached only in severe uncompensated acidosis, while a reaction of pH^8 is attained perhaps only after the administration of large doses of alkalies.

Since the electro-chemical methods of determining the reaction of the blood are too complicated for clinical work the attempt has been made to find a color indicator which would show definite progressive and constant color changes for each degree of the H-ion concentration.

MARRIOT, LEVY, AND ROWNTREE'S METHOD FOR THE DETERMINATION OF THE HYDROGEN-ION CONCENTRATION OF THE BLOOD.[78]

Levy, Rowntree and Marriott used phenolsulphonphthalein which exhibits definite variations in color corresponding to very minute differences in hydrogen-ion concentration between $pH^{6.4}$ and $pH^{8.4}$. They prepared a series of standard solutions of known pH colored by this indicator, and then added an equal amount of this indicator to the solution to be tested and determined which of the colors of the standard series it most closely matched.

Since both blood and serum possess color and since proteins interfere with the colors of many indicators and it is impossible to apply the method directly to the blood, they dialyzed the serum or plasma of the whole, or of the defibrinated, blood in collodion sacks for 5 minutes, added the indicator to the dialysate and matched the resulting color to the standard series. This method while still in use has not proven accurate enough to win recognition.

The method more generally in use is:

VAN SLYKE'S METHOD FOR THE DETERMINATION OF THE CARBON DIOXIDE COMBINING POWER OF THE BLOOD PLASMA. Twenty-five cubic centimeters of blood drawn from the vein are allowed to run from the needle directly into a small chemical bottle which contains 10 drops of a 20% solution of potassium oxalate. (The oxalate used should be dried in the oven over night at 100°C. before the solution is made.) The bottle is quickly closed and shaken vigorously until perfect fluidity of the blood has been obtained. It is then at once centrifugalized, the clear serum pipetted off and placed in a separating funnel of about 300 c.c. capacity. While hemolysis should be avoided as much as possible by immediate centrifugalization yet slight hemolysis will not appreciably affect the results. To determine its alkaline reserve, the plasma is saturated with carbon dioxide at alveolar tension by the operator who blows vigorously through a bottle containing glass beads into the separating funnel, as shown in Fig. 126. If one were to blow directly into the funnel enough moisture would condense on its walls to dilute appreciably the plasma. The funnel should be closed at the stop-cock *S* and with the stopper *T* just before the stream of breath stops and shaken for 1 minute in such a manner that the plasma will be distributed as completely as possible on its walls. After this shaking more alveolar air is blown through the beads into the funnel and the shaking repeated for 1 minute.

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[78] Levy, Rowntree, and Marriott: Arch. of Int. Med., 1915, vol. xvi, p. 389.

The CO_2 apparatus (Fig. 127) is held in a strong clamp *W*, which is lined with rubber, and the lower stop-cock is supported by an iron rod which also is covered with soft rubber tubing. The apparatus, which should be thoroughly cleaned before the determination is started, is completely filled with mercury. Care should be taken that capillaries *A* and *F*, which are above the upper stop-cock, also are filled with mercury and that there are no air bubbles anywhere within the apparatus. This can be tested by lowering and raising the bulb, thus allowing the mercury to run down and up until there is a not a single air bubble in the apparatus. Six dropping bottles, which contain the following solutions, should be at hand:

1. Distilled water. 2. Phenolphthalein (1% in 95% alcohol). 3. Normal ammonium hydroxide. 4. Caprylic alcohol. 5. Normal sulphuric acid. 6. Mercury.

The mercury leveling bulb *H* should be hung by a wire on the extension *N* about on the level with the lower cock *J*. One drop of phenolphthalein and a drop or two of the ammonium hydroxide are then placed in the upper cup *B*. About ½ c.c. of distilled water is then added and then all drawn off except about 2 drops of the alkaline solution.

One cubic centimeter of the saturated plasma is now introduced into the cup *B* and allowed to flow under the alkaline solution so that none of the carbon dioxide will escape. The stop-cock *C* is then turned so that the tubes *E* and *Z* are connected and the plasma allowed to run in until the capillary *F* is exactly filled. Distilled water, 0.5 c.c., is now measured into cup *B* and allowed to run down to the capillary *F*. This is repeated, care being taken that no air enters the apparatus with the liquid. Next 1 drop of caprylic alcohol is admitted into the capillary *F* to prevent foaming, and then about 1.5 c.c. of the sulphuric acid poured into the cup. Enough of the acid is next admitted into the apparatus, carrying the caprylic alcohol along with it, so that the total volume reaches exactly to the 2.5 c.c. mark. The excess of sulphuric acid is now drawn off. A few drops of mercury are now placed in the cup *B* and allowed to flow down to the capillary *F* in order to seal this and make it capable of holding an absolute vacuum. During this whole operation the lower stop-cock *J* should remain open and, when the apparatus is set up, it should be in such adjustment that if the wire *I* which is connected to the bulb *H* is lowered to the hook *O* the mercury will run to the middle of the small bulb just above the fork but not into the fork *Y*. The wire *I* is now placed on the hook *O* and the mercury allowed to fall until its meniscus has dropped to the 50 c.c. mark on the apparatus. This is controlled by the stop-cock *J*. The bubbles of CO_2 will now be seen escaping.

In order to extract the carbon dioxide completely the apparatus is removed from the clamp and shaken by turning it upside down about a dozen times. (The thumb should be placed over cup *B* so as not to lose any of the mercury.) The apparatus is then replaced, the mercury leveling bulb *H* still being at the low level *O*, and the solution allowed to flow into the small bulb below the lower stop-cock (right side). The solution is drained out of the portion of the apparatus above the stop-cock *J* as completely as possible, but without removing any of the gas (the last drop being allowed to remain above). The mercury bulb *H* is now raised in the left hand and with the right hand the lower stop-cock *J* is immediately turned so that the mercury is admitted to the upper part of the apparatus through the left-hand entrance of the stop-cock without readmitting the watery solution. The bulb *H* is now held beside the apparatus so that its mercury level corresponds to that in the apparatus and the gas in the latter is under atmospheric pressure. A few hundredths of a cubic centimeter of water will float on the mercury in the apparatus but this may be disregarded in leveling. The calculation of the result in terms of volume percentage of carbon dioxide bound as carbonate by the plasma is quite complicated and so Van Slyke recommends that we consequently use the direct reading from the apparatus minus 0.12.

The plasma of normal adults yields from 0.65 c.c. to 0.90 c.c. of gas which is the direct reading on the apparatus. If 0.12 is subtracted, the normal figures would be from 53 to 78 in terms of volume per cent. of carbon dioxide chemically bound by the plasma. Figures lower than 50% in adults would indicate acidosis. The results thus obtained give approximately (within 2 or 3%) the volume per cent. of carbon dioxide bound by the plasma.

Example.—The reading on the Van Slyke apparatus is, *e. g.*, 0.74. This minus 0.12 equals 0.62% of carbon dioxide bound by 1 c.c. of plasma, or, for 100 c.c., 62% which is normal.

THE DETERMINATION OF THE ALKALI RESERVE OF THE BLOOD PLASMA.—Marriott[79] has recently published a method of determining the hydrogen-ion concentration of the dialysate of blood serum after the removal of the carbon dioxide which is more accurate and helpful than the preceding test, since it serves for the detection and accurate quantitative estimation of the degree of an acidosis.

The apparatus required includes: a set of tubes containing standard phosphate mixtures; a solution of phenolsulphonphthalein in 0.8% sodium chloride; collodion sacks; a pipette to measure 0.5 c.c.; small test tubes for dialyzing and aerating; an atomizer bulb; a glass tube or pipette drawn out to a fine capillary point and a box for comparing colors.

A 1/15 molecular acid, or primary, potassium phosphate is made by dissolving 9.078 gms. of the pure recrystallized salt (KH_2PO_4) in freshly distilled water, exactly 200 c.c. of a 0.01% solution of phenolphthalein is added and the solution made up to 1 liter.

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[79] Arch. Int. Med., June, 1916, vol. xvii, p. 840.

One-fifteenth molecular alkaline, or secondary, sodium phosphate solution is made by exposing the pure recrystallized salt ($Na_2HPO_4.12H_2O$) protected from dust to the air for from 10 days to 2 weeks, (Ten molecules of water of crystallization will be given off yielding a salt of the formula $Na_2HPO_4.2H_2O$), dissolving 11.876 gms. of this in freshly distilled water, adding exactly 200 c.c. of a 0.017% solution of phenolphthalein and making the solution up to 1 liter. The solution should give a deep rose-red color with phenolphthalein. If only a faint pink color is obtained the salt is not sufficiently pure. The exact amount of indicator is immaterial, provided the same amount is added to each of the phosphate solutions. A small crystal of thymol also is added to each to prevent the growth of moulds. These solutions should be preserved in Jena or Non-sol glass vessels. These solutions are mixed in the proportions indicated below to obtain the desired pH.

pH	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6
Primary sod. phos., c.c.	37.0	27.0	19.0	13.2	8.8	5.6	3.2	2.0	1.0
Secondary sod. phos., c.c.	63.0	73.0	81.0	86.8	91.2	94.4	96.8	98.0	99.0

These solutions are measured into small test tubes, approximately 100 mm. long by 8 mm. internal diameter, made of glass that does not readily give off alkali and these stoppered or sealed. They should be kept in a dark place when not in use. Under these conditions, the solutions will retain their colors for long periods of time.

The salt solution is made up by dissolving 8 gms. of chemically pure sodium chloride in distilled water, adding 220 c.c. of 0.017% phenolsulphonphthalein solution and making the whole up to 1 liter with distilled water. The intention is that this solution shall have just the same strength of indicator as the 2 phosphate solutions, but since a certain amount of that in the salt solution is lost during the dialysis by passing into the sack it is made to contain an initial excess of 10%. To test this solution for free alkali and acids other than carbonic, a little of it is boiled (or aerated with a current of air that has been freed from carbon dioxide by passing through a strong solution of sodium hydroxide) for a minute or so in a test tube of hard Jena glass (which will give off no alkali) in order to expel the carbonic acid. It is then cooled quickly under the tap and compared with the phosphate standards. Its reaction should be 7.0. If not, it may be corrected by the addition to the whole solution of a few drops of very dilute acid or alkali. This solution should be kept in a vessel of Jena or Non-sol glass, or in a vessel of ordinary glass that has been well paraffined on the inside.

The determination must be carried out in a room free from acid and ammonia fumes. While serum, oxalated plasma, or blood may be used, serum is to be preferred since the addition of oxalate, unless exactly neutral, will introduce a source of error. The blood should be collected in a small tube and the serum separated as quickly as possible, preferably by centrifuging, otherwise there will be a passage of some of the alkali of the plasma to the cells following the loss of some carbon dioxide. Hemolysis must be avoided.

Exactly 0.5 c.c. of serum is pipetted into one of the small collodion sacks, which has previously been washed inside and out with the salt solution. (In washing the sack, no part but the top edge should be touched with the fingers. The sack is emptied by tipping it with a clean glass rod or with a microscopic slide. Sacks may be used more than once, providing they are thoroughly washed with salt solution after each test.) The sack is then lowered into a small test tube, approximately 8 mm. internal diameter and 50 mm. long, containing 2 c.c. of the indicator salt solution. The level of the fluid on the outside of the sack should at least be as high as that on the inside. At the end of 7 minutes the sack is removed and the dialysate transferred to a clean test tube from 100 to 140 mm. long and having the same diameter as the tubes containing the phosphate standards. A rapid current of air is now bubbled through the solution in order to remove the carbon dioxide. This may be done with an atomizer bulb connected with a narrow glass tube drawn out to a capillary point. (Troublesome foaming may be prevented by adding a drop of octyl alcohol or toluol.) The blowing is continued for 3 minutes and then the color in the tube is compared with that of the standard phosphate tubes. The reading is a measure of the reserve alkalinity which is expressed as RpH.

In the case of every normal person on a general mixed diet the RpH of the serum was found to be 8.5±0.05. A normal adult's serum drawn after a fast of 16 hours gave a reading of 8.35. The serum of normal infants under 1 year of age frequently gives values as low as 8.3. This accords well with the observation that the carbon dioxide tension in the alveolar air is lower, that the combined carbon dioxide of the plasma is less and that the ammonia coefficient in the urine is often higher in infants than in adults. This slight but normal acidosis might well be the result of the more active metabolism of infants, leading to a proportionately greater production of acids, although the fact that infants' blood is usually obtained for this test by cupping should not be overlooked.

In all the cases of acidosis studied (including cases of nephritis and diabetes in adults, and nephritis, recurrent and idiopathic acetonemia and severe diarrheas in children. The diarrheal cases were of the type described by Howland and Marriott) the RpH of the serum showed deviations from the normal. The more severe the acidosis (as judged by other methods) the lower the RpH. Especially striking was the parallelism between alveolar carbon dioxide tension and the RpH in all cases without any disturbance of the respiratory center or lesion of the pulmonary epithelium.

The results obtained by Van Slyke's method of determining the carbon dioxide combining power of the blood plasma (see page 533) were in a general way proportional to the RpH of the serum. In cases of alkali starvation the RpH gave information as to the probable amount of alkali needed to replenish the reserve while a determination following the administration of alkali showed whether or not the amount given was sufficient.

The values obtained for the RpH of the serum may, in the light of Marriott's experience, be interpreted as follows: Values for RpH of from 8.4 to 8.55 correspond to alveolar carbon dioxide tensions of from 38 to 45 mm. and are to be considered as normal values for adults. Values between 8.0 and 8.3 correspond to alveolar carbon dioxide tensions of from 28 to 35 mm. and indicate a moderate degree of acidosis.

When the value for RpH is as low as 7.7, corresponding to an alveolar carbon dioxide tension of 20 mm., the individual is in imminent danger. During coma an RpH as low as 7.3, corresponding to an alveolar air tension of 11 mm. was observed. In infants under one year of age a value for RpH of8.3, corresponding to 35 mm. tension in the alveolar air, is not to be considered abnormal.

It has been Marriott's experience that unless the RpH of the serum is below 7.9 the acidosis may be successfully combated by dietetic regulation or by the administration of alkali by mouth. When the RpH of the serum falls below 7.9 intravenous administration of alkali is usually indicated.

Löwy Method.—Löwy's method is still the best for determining the total alkalinity of the blood. Into a special flask, on the neck of which are marks indicating 45 and 50 c.c., are measured 45 c.c. of 0.25 % ammonium oxalate. To this are added 5 c.c. of blood. The blood is therefore laked and will not coagulate, hence the alkalinity determined is that of the total blood. This is then titrated with *0.04N* tartaric acid using lacmoid paper saturated with magnesium sulphate as indicator. By this method it has been determined that 100 c.c. of normal blood contains from 400 to 600 mgms. of NaOH.

Salkowski's Method.—Salkowski's method is certainly simple. A known amount of ammonium sulphate is mixed with the blood and the ammonia set free determined by Schlösing's method (see page 123).

Under a bell-jar is placed a dish containing 20 gms. of finely pulverized ammonium sulphate dissolved in 20 c.c. of water. In a receptacle above this is 10 c.c. of *0.25N* H_2SO_4. Into the lower dish is poured 10 c.c. of the blood to be examined (the measuring-glass used for the blood is first washed in 1% sodium oxalate solution to prevent coagulation). The blood is mixed with the ammonium sulphate solution and the apparatus covered at once with the bell-jar. In 5 or 6 days all of the ammonia set free from the ammonium sulphate will have been taken up by the sulphuric acid and its amount may be determined by titrating this. By this method the alkalinity for men has been found to be from 350 to 400 mgms. and for women from 300 to 350 mgms. of NaOH per 100 c.c. of blood.

SELLARD'S METHOD (QUALITATIVE) FOR DETERMINING THE TITRATABLE ALKALINITY OF THE BLOOD.[80] One cubic centimeter of serum is shaken up thoroughly with 25 c.c. of absolute alcohol and filtered into an evaporating dish. Perfectly dry apparatus should be used. Without washing the precipitate 3 or 4 drops of an 0.5% solution in absolute alcohol of phenolphthalein are added to the filtrate and this evaporated to dryness on a steam bath (at not over 100°C).

In the case of normal serum the residue is continuously red. In the presence of an acidosis the residue is colorless or pink but on the addition of 1 drop of water becomes an intense red. In case of an extreme acidosis it remains colorless even after the addition of water.

To prove the neutrality of the absolute alcohol 0.1 c.c. of $0.005N$ NaOH is added to 25 c.c. of the alcohol, also 2 or 3 drops of phenolphthalein as indicator, and the specimen evaporated to dryness. The residue should be distinctly pink.

CHLORIDES OF BLOOD

To determine the chlorides of the blood 3 c.c. of the blood to be examined are measured with a pipette into a 50 c.c. graduated centrifuge tube, 15 c.c. of $0.01N$ acetic acid added and the specimen diluted to 30 c.c. with distilled water. The tube is then placed in a beaker of boiling water to bring about the coagulation of the protein, care being taken that the contents of the tube are agitated occasionally with a stirring rod. After the protein has coagulated the tube is cooled, again made up to volume (30 c.c.) and centrifuged. The slightly colored supernatant fluid is now poured into a dry centrifuge tube, about 6 drops of a strong solution of colloidal iron added and the tube placed in a beaker of hot water for a few minutes. This will completely precipitate all the protein. The specimen is then centrifugalized or filtered once more and 10 c.c. (equivalent of 1 c.c. of blood) of the clear fluid poured into a 25 c.c. volumetric flask together with 10 c.c. of the standard silver nitrate solution[81] and 1 c.c. of the ferric alum indicator[82] added.

The fluid is now made up to volume and shaken thoroughly, centrifugalized in a large (50 c.c.) centrifuge tube and the clear supernatant fluid decanted. One then titrates 20 c.c. of this fluid, which is the equivalent of 0.8 c.c. of blood, with a standard ammonium thiocyanate solution[83] of the same strength as the silver nitrate, until a distinct yellow color shows throughout the mixture. The titration result, divided by 0.8, subtracted from 10, to obtain the silver nitrate used, and multiplied by .001 and again multiplied by 100, gives the percentage in the blood of chlorides as sodium chloride.

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[80] Bull. of the Johns Hopkins Hosp., Apr., 1914, vol. xxv, p. 101.

[81] This standard is prepared by dissolving 2.906 gms. of silver nitrate in distilled water and making up to 1 liter, therefore 1 c.c. = 0.001 gm. of sodium chloride.

[82] This indicator is made by dissolving 100 gms. of crystalline ferric ammonium sulphate in 100 c.c. of 25% nitric acid.

[83] Standard ammonium thiocyanate solution is prepared by dissolving 1.3 gms. of ammonium thiocyanate in 800 c.c. of water, titrating against the above silver nitrate standard and ascertaining the amount of water which must be added to the solution to make each 1 c.c. of it equivalent to 1 c.c. of the standard silver nitrate solution; that is, to indicate 0.001 gm. of sodium chloride.

Example.—Suppose the reading on burette is 3.2 c.c. This divided by $0.8 = 4$: $10 - 4 = 6$: $6 \times .001 \times 100 = 0.6$, the percentage of chlorides (as NaCl) in this specimen of blood.

NITROGENOUS BODIES OF THE BLOOD

Total Nitrogen.—To determine the total nitrogen of the blood exactly 1 c.c of the specimen to be examined is measured into a long-necked Jena glass Kjeldahl flask, 20 c.c. of concentrated sulphuric acid and about 0.2 gm. of copper sulphate added and the mixture boiled in the digestion rack for about 1 hour after it has become colorless. The flask is then cooled and the contents diluted with about 200 c.c. of ammonia-free water. Then one adds about 40 c.c. of a saturated sodium hydroxide solution, that is, a little more than is necessary to neutralize the sulphuric acid, and a little coarse pumice stone or a few pieces of granulated zinc to prevent thumping and a small piece of paraffin to lessen the tendency to froth. The flask is then connected by means of a safety tube with a condenser so arranged that the delivery tube passes into a vessel containing a known volume (the volume used depending upon the nitrogen contents of the blood) of $0.1N$ H_2SO_4 to which has been added a few drops of congo red. (Congo red 0.5 gm. dissolved in a mixture of 90 c.c. of distilled water and 10 c.c. of 95% alcohol.) The end of the delivery tube must reach beneath the surface of the fluid and the tube should be of a large enough caliber to avoid the sucking back of the fluid. The contents of the distillation flask should be mixed very thoroughly by shaking (or rotating) and the mixture then distilled until about ⅔ of the solution has passed over. The partly neutralized $0.1N$ H_2SO_4 is now titrated against $0.1N$ NaOH. In this way one determines the amount of $0.1N$ H_2SO_4 neutralized by the ammonia which has distilled over. 1 c.c. of $0.1N$ H_2SO_4 is the equivalent of 0.0014 gm. nitrogen. This multiplied by 100 is the amount of N in 100 c.c. of blood.

Folin-Farmer Microchemical Method.—Exactly 1 c.c. of the blood to be examined is pipetted into a 25 c.c. volumetric flask, diluted with distilled water up to the 25 c.c. mark and well mixed. One cubic centimeter of the diluted blood is pipetted into a test tube of such a size that it will slip into the aeration apparatus. From 0.1 to 0.3 gm. of potassium sulphate, a drop of 10% copper sulphate solution and 1 c.c. of concentrated sulphuric acid are added in the order named and the digestion carried out as in the determination of non-protein nitrogen (see page 540). The result obtained above is for $\frac{1}{25}$ c.c. of blood.

Example.—Suppose the dilution was to 50 and the reading 75 which on the scale would indicate 0.56 mgm. had the dilution been to 100. Therefore $0.56 \div 2 = 0.28$ mgm., in 2 c.c. of blood or 14 mgms. in 100 c.c.

To calculate the amount of urea which a given amount of nitrogen would represent the value of the latter is multiplied by the factor 2.14.

Non-proteid Nitrogen of the Blood.—The non-proteid nitrogenous products of catabolism which are constituents of the blood and which may accumulate in cases of renal insufficiency are:[84] Urea, formed largely in the liver from the ammonia resulting from the deaminization of amido acids set free in digestion but not of immediate use to the animal organization; uric acid, a product of the enzymatic transformation of the amino- and oxypurins, in which various glands of the body participate; and creatinin, which would appear to be formed in the muscle tissue from creatin. Under normal conditions the percentage relationship of these bodies to total non-proteid nitrogen is: urea 50%, creatinin 2%, uric acid 2%, ammonia 0.3% and undetermined nitrogen 4.6%. In the urine these figures are: urea 85%, creatinin 5%, uric acid 1.5%, ammonia 4% and undetermined nitrogen 4.5%. Judged by their comparative composition the kidney concentrates the creatinin 100 times, the urea 80 times and the uric acid only 20 times. Creatinin is therefore of these 3 bodies the most readily eliminated, urea next and uric acid least.

To determine the non-proteid nitrogen 5 c.c. of the blood to be examined are measured into a 50 c.c. volumetric flask containing about 35 c.c. of 2.5% trichloracetic acid and the volume then made up to 50 c.c. with this same acid. The flask is then shaken vigorously. At the end of 30 minutes (or as soon after as convenient) the specimen is filtered through a dry filter. To the filtrate are added about 2 gms. of kaolin and it is shaken vigorously. The mixture is then allowed to stand for from 5 to 10 minutes and the filtration repeated. This filtrate should be quite colorless. One next pipettes 10 c.c. of the filtrate (the equivalent of 1 c.c. of blood) into a test tube about 200 mm. long and of a sufficient diameter to slip into a 100 c.c. graduated cylinder (see Fig. 27). From 0.1 to 0.3 gm. of potassium sulphate, a drop of 10% copper sulphate and 1 c.c. of concentrated sulphuric acid are added in the order named (these reagents should be of the highest purity). This is then boiled over a microburner, at first gently, until a dark brown color appears. Gradwohl and Blaivas recommend that the solution then be allowed to cool and a drop of peroxide of hydrogen added. If the mixture does not clear it is heated gently over the microburner and, if that is not sufficient, this process is repeated. The tube is now allowed to cool and about 5 or 6 c.c. of distilled water added.

As a means of removing the fumes, the suction pump is connected by a 2-hole stopper to a large bottle (Fig 128) containing a solution of sodium hydroxide. The tube *B* should be attached to a funnel held over the mouth of the test tube *D*. After a few determinations have been made, it is well to wash the funnel to remove any acid which may have condensed upon it.

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[84] Quoted from Myers, The Jour. of Lab. and Clin. Med., Apr., 1920, v, p. 418.

Aeration is carried out exactly in the manner for urea, the only difference being that saturated sodium hydroxide is used instead of saturated sodium carbonate. The same table is used for the calculations.

Blood Urea.—Marked urea retention occurs in a wide variety of conditions: in the terminal stages of chronic interstitial nephritis, in some cases of acute nephritis (but it is not at all marked in parenchymatous nephritis), in bichloride and in lead poisoning, in congenital cystic kidney, malignancy, pneumonia, intestinal obstruction and sometimes in syphilis and cardiac conditions. A normal urea with a high uric acid content in the blood is of value in the diagnosis of gout. In eclampsia the blood urea is but slightly elevated if at all, while in normal pregnancy the figures are low.

The blood urea will vary from the normal only late in a case of renal insufficiency, much later than creatinin and uric acid, and its determination is of little value in diagnosis but is of value in treatment. If increased blood urea may be reduced by increasing the fluid intake and decreasing the proteid of the diet.

Case 9559, a woman 45 years old, was admitted on May 3, 1920. Two weeks previous to this time her blood creatinin was 2.4 mgms. per 100 c.c. and her phenolsulphonephthalein output 41% in 2 hours. On May 5 the blood creatinin was 3.29 mgms., the blood urea 35.04 mgms. per 100 c.c., and the phenolsulphonephthalein output 25%. On May 13 the blood creatinin was 3.97 mgms. and the blood urea 30 mgms. On May 22 the blood creatinin was 4.11 mgms. and the blood urea 145.2 mgms. On May 25, the blood creatinin was 14.12 mgms. and blood urea 350.4 mgms. per 100 c.c. Then came the first convulsion. She died May 28, 1920, in uremia.

Urea.—For the determination of urea 2 c.c. of the blood to be examined are measured with an Ostwald pipette into a test tube which already contains 2 c.c. of distilled water and 0.1 gm. of urease. This tube should be of such size that it will readily slip into a 100 c.c. graduated cylinder. This is then incubated for ½ hour in a beaker of water at 50°C. At the end of this time 2 drops of caprylic alcohol or 1 c.c. of amylic alcohol are added to prevent foaming in the subsequent aeration. The urease converts the urea into ammonium carbonate. The ammonia is then liberated by an excess of sodium carbonate and carried over by suction into hydrochloric acid where it forms ammonium chloride. The ammonia may now be determined colorimetrically by the use of Nessler's reagent. The apparatus is that used for the determination of urea in the urine (see page 113)

Into cylinder 1 are poured 20 c.c. of distilled water and 2 to 3 drops of 10% HCl. This cylinder is now closed and cylinder 2 opened. To the test tube containing the digested blood an equal volume of saturated sodium carbonate is allowed to run slowly down under the blood, this placed at once in the cylinder and this immediately closed, with the long tube dipping practically to the bottom of the fluid in the test tube and the connection carefully sealed. The suction by a Chapman pump is slow for about 5 minutes and then gradually increased to as much as the apparatus will stand. The aeration is kept up for from 30 to 45 minutes. At the end of this time the pump is disconnected, the rubber stopper from cylinder 1 removed and the tube washed off with 2 or 3 c.c. of distilled water.

The color test is made as follows. Into a 50 c.c. volumetric flask is pipetted 5 c.c. of ammonium sulphate solution containing 1.0 mgm. of nitrogen per 5 c.c. of the solution (this is the standard solution) (see page 109) and then 25 c.c. of distilled water and 20 c.c. of Nessler's solution (see page 109) diluted 1 to 5 added.

To cylinder 1, containing the ammonia in the form of ammonium chloride, add from 10 to 20 c.c. of diluted Nessler's solution (1 to 5), depending upon the depth of color, and then dilute to 50 c.c., 100 c.c., etc., according to the color. The colorimetric reading should be made at once and computed from the following table:

TABLE IV*

ESTIMATION OF NITROGEN WITH THE HELLIGE COLORIMETER

Colorimetric reading	Nitrogen mgms. per dilution of 100 c.c.	Colorimetric reading	Nitrogen mgms. per dilution of 100 c.c.	Colorimetric reading	Nitrogen mgms. per dilution of 100 c.c.
20	1.73	40	1.31	60	0.89
21	1.71	41	1.29	61	0.87
22	1.69	42	1.27	62	0.85
23	1.67	43	1.25	63	0.83
24	1.65	44	1.23	64	0.81
25	1.62	45	1.20	65	0.78
26	1.60	46	1.18	66	0.76
27	1.58	47	1.16	67	0.74
28	1.56	48	1.14	68	0.72
29	1.54	49	1.12	69	0.70
30	1.52	50	1.10	70	0.67
31	1.50	51	1.08	71	0.65
32	1.48	52	1.06	72	0.63
33	1.46	53	1.04	73	0.61
34	1.44	54	1.02	74	0.59
35	1.41	55	0.99	75	0.56
36	1.39	56	0.97	76	0.54
37	1.37	57	0.95	77	0.52
38	1.35	58	0.93	78	0.50
39	1.33	59	0.91	79	0.48

*Myers and Fine: Table copied from Gradwohl and Blaivas.

Ammonia.—In the determination of urea any preformed ammonia would be determined at the same time. As a correction for the urea determination necessary in some cases, and also if the ammonia is to be determinated separately, a specimen of blood is examined exactly as above except that no urease is used. The blood should be examined immediately after it is drawn from the vein since on standing the ammonia begins at once to increase.

Under normal conditions [85] the ammonia of the blood varies from 0.4 to 0.75 mgms., never more than 1 mgm., per 100 c.c. An amount of 3 mgms. or over per 100 c.c. may certainly be considered abnormal. Normally, diet has no appreciable influence on the ammonia although for the sake of uniformity the patients are placed for at least 1 day on a diet consisting of at least 32 ounces each of milk and albumin water per 24 hours.

McNeil and Levy found that an increased ammonia content of the blood could not in all cases be explained by the presence of an acidosis, nor was it nearly as common in cases with advanced hepatic disease as one would expect. In one man with periodic attacks of nausea and vomiting an ammonia content of 23 mgms. per 100 c.c. of blood was found. The ammonia was not increased in the blood of four patients with uremia, but was in several cases of eclampsia.

Uric Acid in the Blood.—Under normal conditions the blood contains from 1 to 3.5 (average 2) mgms. of uric acid per 100 c.c. This is increased physiologically during the first 3 or 4 days of life and pathologically in gout, lead and mercuric bichloride poisoning, malignancy, acute infections especially pneumonia, in leukemia and in chronic insterstitial (not parenchymatous) nephritis. These increases may be due to increased production, as in leukemia, or to decreased elimination, as in nephritis. In very early stages of interstitial nephritis, before any concentration in the blood of urea or creatinin can be demonstrated, that of uric acid may be very definite, which makes this an early diagnostic sign of this disease and perhaps the most delicate index of renal function we have. [86] Later, after the renal insufficiency has become marked, the uric acid content may reach 10, 15 and in one case just before death 27 mgms. per 100 c.c. of blood. In gout the uric acid of the blood is invariably increased, ranging from 3.5 to 5.5 mgms. and even 9 mgms. per 100 c.c. of blood. (The reader will have in mind the common, perhaps invariable, association of chronic interstitial nephritis with gout. Indeed the old term applied to the small contracted kidney was "gouty kidney." It is an old idea now again advanced that gout is merely a form of chronic interstitial nephritis with conspicuous joint complications.[87])

In other forms of arthritis, excepting those with a definite renal complication the uric acid varied from 1.6 to 3.6 mgms. (the majority below 3 mgms.) per 100 c.c. of blood.

For the estimation of uric acid in the blood [88] 10 c.c. of the specimen to be examined are measured into a casserole of at least 375 c.c. capacity and 50 c.c. of $0.01N$ acetic acid added.[89] The casserole is placed on a water-bath and heated until coagulation takes place, then over a free flame until the contents come to a boil, stirring continuously. About 1 spoonful (4 c.c.) of alumina cream [90] is now added and the boiling continued for one minute, stirring continuously. The specimen is now filtered and the coagulum on the filter paper washed back into the casserole with about 100 c.c. of hot distilled water. This mixture in the casserole is next heated to the boiling point over a free flame and then filtered. The combined filtrates are evaporated by boiling, then slowly over a free flame, until their volume has been reduced to about 500 c.c., then in the water bath until it is evaporated down to 1 or 2 c.c. This is then transferred to a conical centrifuge tube of 15 c.c. capacity, washing the casserole with 2 or 3 portions of hot water but keeping the final volume below 10 c.c. When this has cooled, 15 drops of ammoniacal-silver-magnesium mixture (see page 119) are added, the tube is shaken and placed in a refrigerator for about 15 minutes (to allow the precipitation of purine). It is then centrifugalized for from 3 to 5 minutes and the supernatant fluid then poured off by inverting the tube and wiping its lip with filter paper. The ammonia of the sediment is then removed by suction. This is accomplished by attaching the centrifuge tube to the rubber tubing of the Chapman pump.

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[85] McNeil and Levy, Jour. of Lab. and Clin. Med., 1917, iii, 18.

[86] See Myers, Fine and Lough, Arch. of Int. Med., 1916, vol. xvii, p. 570.

[87] Jour. A. M. A., 1916, lxvi, 2051.

[88] Benedict's method as given by Gradwohl and Blaivas.

We are now ready for the development of color and for the reading. The beginner should work as fast as possible as the color may fade or turbidity may develop.

A 100 c.c. graduated cylinder is now prepared for the unknown and a 50 c.c. volumetric flask for the uric acid standard solution. Then 5 c.c. of uric acid standard (see page 545) (5 c.c. = 1 mgm. of uric acid) are measured with a pipette into the 50 c.c. volumetric flask. To the uric acid standard 2 drops of a 5% solution of potassium cyanide, 2 c.c. of Folin-Macallum reagent (see page 120) and 20 c.c. of saturated sodium carbonate are added and, in 1 minute, water, to the 50 c.c. mark. To the precipitate in the centrifuge tube one now adds 2 drops of a 5% potassium cyanide solution (the tube is shaken so as to dissolve the precipitate), 2 c.c. of the Folin-Macallum reagent and then washes the contents of the centrifuge tube into a 100 c.c. graduate with from 15 to 20 c.c. of saturated sodium carbonate. More carbonate, *i.e.*, 20 c.c., is used when the color is stronger than the standard and 15 c.c. when it is weaker, so that the unknown solution may be paler in color than the standard solution. From 40 to 60 seconds should now be allowed to pass before determining how much to dilute this.

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[89] The $0.01N$ acetic acid is prepared by adding 0.6 c.c. of glacial acid to 1 liter of distilled water. This should be made up fresh at least each 2 weeks.

[90] For the preparation of alumina cream 500 c.c. of 8% aluminum acetate in acetic acid are precipitated with sodium bicarbonate (dry) until the solution is neutral to litmus paper. This is allowed to stand for 24 hours and the supernatant fluid decanted. This is repeated 6 times. On the sixth day the precipitate is filtered and stored in a jar with the addition of 5 c.c. of chloroform. It is now ready for use. It should be kept in the ice-box for storage.

It is then diluted with distilled water to 25, 50 or 100 c.c., depending upon the depth of color obtained. Table II gives the data for working out the amount of uric acid present.

Example.—Suppose the final dilution of the unknown was 25 and the reading was 42. 42 in the table is equivalent to 1.24 mgms. This is divided by 4 because it is ¼ as strong as the amount in the table (*i. e.*, of 100) which equals 0.31 mgm. in 10 c.c. of blood (which is the amount of blood we started with). In 100 c.c. of blood we would have 10×0.31=3.1 mgms. of uric acid.

Myers' Modification of Folin and Wu's Method.—To 5 c.c. of well-mixed oxalated blood in a 100 c.c. Erlenmeyer flask (or 100 c.c. glass stoppered cylinder) are added 35 c.c. of water (7 volumes), 5 c.c. of 10% sodium tungstate and finally 5 c.c. of exactly *0.75N* sulphuric acid, rotating the flask all the time and then shaking thoroughly. (There are some advantages in using 7 c.c. of blood since one secures rather quickly 40 c.c. of filtrate, the equivalent of 4 c.c. of blood, but satisfactory results may be obtained with but 2.5 or 3 c.c. of blood.) When the blood is properly coagulated the color of the coagulum will turn from pink to brown. If this change does not occur the coagulation is incomplete, due probably to too much oxalate, in which case 5% sulphuric acid may be added a drop at a time, shaking between each addition, until the coagulation is complete. Folin and Wu caution against an excess of sulphuric acid since this apparently precipitates some of the uric acid. If the mixture is now carefully poured upon the double portion of a filter just large enough to hold the mixture, the filtrate will probably come through perfectly clear; if not, the first portion may be returned to the filter. To prevent evaporation a watch glass may be placed over the top of the funnel. It is convenient to filter into a 50 c.c. graduated cylinder.

When the filtration is complete the volume of the filtrate is recorded (ordinarily between 25 and 30 c.c.) and it is poured into a 50 c.c. centrifuge tube. Five cubic centimeters of 5% silver lactate solution in 5% lactic acid are added, the fluid stirred and centrifuged. The supernatant fluid is then poured off. To the precipitate is added about 3 c.c. of 10% sodium chloride in *0.1N* hydrochloric acid (prepared by adding 1 c.c. of concentrated hydrochloric acid to 100 c.c. of 10% chloride solution). It is then stirred with a glass rod. One now adds 3 to 4 c.c. of water, 1 drop of 5% potassium cyanide, stirs again and centrifuges. This treatment sets the uric acid free from the precipitate. The supernatant fluid is then poured into an accurately graduated 25 c.c. cylinder.

Into a similar 25 c.c. cylinder one introduces with an Ostwald-Folin pipette 1 c.c. of standard uric acid solution,[91] then 4 c.c. of the acidified sodium chloride solution and sufficient water to make the volume approximately the same as that of the unknown. One drop of 5% potassium cyanide is then added.

To the standard is now added 1 c.c. of the uric acid reagent [92] and 0.5 c.c. to the unknown specimen. Then saturated sodium carbonate solution, 5 to 6 c.c., is added to the standard and 3 to 4 c.c. to the unknown. The color is allowed to develop for about 5 minutes. The standard is then diluted to 20 to 25 c.c., and the unknown, if darker than the standard, to a similar depth of color.

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[91] Benedict's standard uric acid solution is prepared as follows: One dissolves 4.5 gms. of pure crystalline hydrogen disodium phosphate and 0.5 gm. dihydrogen sodium phosphate in 200 to 300 c.c. of hot water. This is filtered and made up to about 250 c.c. with hot water. This warm, clear solution is poured on to 100 mgms. of uric acid suspended in a few cubic centimeters of water in a 500 c.c. volumetric flask. This is agitated until completely dissolved. Then at once exactly 0.7 c.c. of glacial acetic acid is added, the volume made up to 500 c.c., mixed and then 5 c.c. of chloroform added. One cubic centimeter of this solution contains 0.2 mgm. of uric acid. This solution should be freshly prepared every 2 months.

[92] Benedict's modification of the Folin-Denis uric acid reagent is prepared by boiling 100 gms. of sodium tungstate, 20 c.c. of concentrated hydrochloric acid and 30 c.c. of 85% phosphoric acid in 750 c.c. of distilled water for 2 hours, preferably under a reflux condenser, and then making the volume up to 1000 c.c. with water.

With this technic fairly deep blue colors are obtained even with normal blood, colors that can readily be matched in the colorimeter. The slight cloud that sometimes appears may easily be separated in the centrifuge. For the calculation the following formula may be used: $\frac{S}{R} \times \frac{X}{25} \frac{0.2 \times 100}{B}$ =mgm. of uric acid in 100 c.c. of blood, in which S represents the depth of the standard (15 mm.), R the reading of the unknown, X the dilution of the unknown, 0.2 the strength of the standard in mgm. and B the number of cubic centimeters of blood to which the filtrate employed was equivalent.

Estimation of Uric Acid with the Test Tube Colorimeter.—The technic described above readily lends itself also to use with the test tube colorimeter.

Method.—Three cubic centimeters of well mixed oxalated blood are pipetted into a large test tube or small flask and 21 c.c. of water added. The precipitation of the proteins is now carried out according to the above described procedure of Folin and Wu, 3 c.c. of the sodium tungstate and 3 c.c. of the $0.75N$ H_2SO_4 being used.

In case it is necessary to add extra sulphuric acid this should be done carefully as an excess may lead to a precipitation of the uric acid.

To 15 c.c. of the filtrate in a 20 c.c. centrifuge tube[93] are added 2 c.c. of 5% silver lactate in 5% lactic acid. After stirring, this is centrifuged. The supernatant fluid is poured off and discarded. To the precipitate is added about 1.5 c.c. of 10% NaCl in $0.1N$ HCl and this stirred with a glass rod. Then 3 to 4 c.c. of water are added, the fluid stirred again and centrifuged. This treatment frees the uric acid from the precipitate. The clear supernatant fluid is poured into the right-hand tube of the colorimeter and one drop of 2.5% potassium cyanide added.

Into the left hand tube of the instrument is introduced 1 c.c. of the standard uric acid solution (diluted 1:5 and therefore containing 0.04 mgm. of uric acid). Now 1.5 c.c. of the 10% NaCl in $0.1N$ HCl is added and sufficient water to make the volume practically the same as that of the unknown. Then 1 drop of 2.5% potassium cyanide is added.

To both tubes now is added 0.3 c.c. of the uric acid reagent and 2 c.c. of saturated sodium carbonate solution. The color is allowed to develop for 3 to 5 minutes, the standard then diluted to 10 c.c. (20 c.c. if the unknown shows a weak color development) and the unknown to the same intensity of color as the standard, adding the water a drop or two at a time as one approaches the end point.

To calculate the number of milligrams of uric acid per 100 c.c. of blood the following formula may be used, in which S represents the strength of the standard (0.2 or 0.4 mgm. to 100 c.c., depending on the dilution), D the dilution in cubic centimeters of unknown required to match the standard, and B the number of cubic centimeters of blood employed. $\frac{S \times D}{B}$ =mgm. uric acid per 100 c.c. of blood. If, for example, 15 c.c. of filtrate are used, the equivalent of 1.5 c.c. of blood, with a standard containing 0.4 mgm. diluted.

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[93] Cylindrical trunnion cups may be substituted for the conical cups of the ordinary centrifuge. In these, test-tubes or special 20 c.c. centrifuge tubes may be employed. The cups work quite as well for the 15 c.c. conical centrifuge tubes.

to 10 c.c., and if the unknown was diluted to 17.1 c.c. to match the standard, the formula will work out as follows:

$$\frac{0.4 \times 17.1}{1.5} = 4.6 \text{ mgms. to } 100 \text{ c.c.}$$

Creatinin in the Blood.—The creatinin of the blood, which is almost exclusively of endogenous formation, furnishes our best criterion as to the excretory power of the kidney (Myers). The blood of normal persons contains from 1 to 2 mgms., as a rule about 1.2 mgms., per 100 c.c. of blood. While from 2 to 3 mgms. is abnormal yet we often find that much in the blood of patients who have no evidence of renal insufficiency. Above 3 mgms. would mean a definite retention, over 4 mgms. would mean considerable impairment of renal function, while cases with 5 mgms. or more seldom show any improvement under treatment and practically all die in a rather limited time unless the renal condition be fairly acute, in which case recovery is possible.[94]

The value in prognosis of creatinin determinations is well illustrated by those patients who with high creatinin content of the blood and with very few months to live yet are up and about, feel well and may show considerable clinical improvement. Some certainly show none of the subjective symptoms usually associated with uremia.

This was well illustrated by J. S., No. 8866, aged 33 years, admitted for "easy fatigability." He was pale, had albuminuric retinitis but practically no subcutaneous edema of any part of body, but for over a year had been rather slow of speech. His blood pressure varied from 180 to 210 mm. Hg., his spinal fluid gave a strong positive Wassermann reaction on all dilutions, his urine contained 2 gms. albumin per liter of urine and casts of all descriptions. His renal functional test, tried several times, gave no elimination of phenolsulphonephthalein in 2 hours and his blood creatinin, always high, was, when he left, 10.9 mgms. per 100 c.c. of urine. He died 10 days later. His doctor reported that he was quite clear mentally up to 1 hour of his death and had had no headache, no drowsiness and very little subcutaneous edema.

For M. L. No. 9559, aged 45, see page 541.

While preparing the specimen of blood for the determination of creatinin the standard solution should be prepared at the same time in order that the color may develop equally fast in both. Otherwise the results will be incorrect.

The blood should be taken at least 14 hours after the last meal, that is, between 7 and 9 o'clock in the morning. Five cubic centimeters of blood measured in an Ostwald pipette are allowed to run to the bottom of a 50 c.c. centrifuge tube into which have previously been measured 20 c.c. of distilled water. The pipette is washed by alternating drawing up and blowing down this blood and water mixture, which is then stirred to lake the cells. Dry picric acid (0.5 gm.) is added to precipitate the protein and the mixture thoroughly stirred. After standing for from 10 to 15 minutes, during which time it is stirred occasionally, the specimen is centrifugalized for 5 minutes at about 1500 revolutions per minute and then filtered through a small filter paper into a clean, dry test tube. To 10 c.c. of this filtrate is added 0.5 c.c. of a 10% sodium hydroxide solution; while to 20 c.c. of a standard creatinin solution [95] is added 1 c.c. of 10% sodium hydroxide. Both are now allowed to stand for 10 minutes and then are read in the colorimeter.

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[94] For Myers' reports of cases with 5 mgms. or more, see Am. Jour. Med. Sci., 1919, clvii, 674, and the Jour. of Lab. and Clin. Med., June, 1920, vol. v, p. 566.

The formula for the computation of the result is as follows: $89 - \text{reading} \times 0.0179 \times 5 =$ the number of mgms. of creatinin per 100 c.c. of blood.

Example.—Let us assume the reading in an experiment is 64. Then $89 - 64 = 25 \times 0.0179 = 0.4475 \times 5 = 2.2375$ mgms.

Slightly less accurate results than these may be obtained by using as standard *0.25 N* bichromate of potash solution (made by dissolving 12.28 gms. of potassium bichromate in distilled water and making the solution up to 1 liter). When using this standard the filtrate is treated as in the preceding and the result is multiplied by 5. In this case Table V should be used.

TABLE V *

ESTIMATION OF CREATININ IN THE BLOOD WITH THE HELLIGE COLORIMETER

Colorimetric reading	Creatinin mgms. per dilution of 100 c.c.	Colorimetric reading	Creatinin mgms. per dilution of 100 c.c.	Colorimetric reading	Creatinin mgms. per dilution of 100 c.c.
40	0.80	57	0.55	74	0.31
41	0.78	58	0.54	75	0.30
42	0.77	59	0.52	76	0.28
43	0.75	60	0.51	77	0.27
44	0.74	61	0.50	78	0.25
45	0.72	62	0.48	79	0.24
46	0.71	63	0.47	80	0.22
47	0.70	64	0.45	81	0.21
48	0.68	65	0.44	82	0.20
49	0.67	66	0.42	83	0.18
50	0.65	67	0.41	84	0.17
51	0.64	68	0.40	85	0.15
52	0.62	69	0.38	86	0.14
53	0.61	70	0.37	87	0.12
54	0.60	71	0.35	88	0.11
55	0.58	72	0.34	89	0.10
56	0.57	73	0.32	90	0.09

* The table here given must be used when N/4 bichromate is used as a standard. Myers and Fine. Table copied from Gradwohl and Blaivas.

Creatin of the Blood.—Under normal conditions the blood contains from 3 to 7 mgms. of creatin per 100 c.c. While this is increased in the last stages of nephritis along with the other non-protein nitrogenous substances, the results of its determination have not as yet proven of value. (Myers.)

For the determination of creatin plus creatinin 1 or 2 c.c. of the filtrate used for sugar or creatinin determinations is measured with an Ostwald-Folin pipette into a small test-tube or a 10 c.c. graduate and autoclaved at 20 pounds pressure for 20 minutes. The solution is then cooled, made up to 8 c.c. with a saturated solution of picric acid and then 0.4 c.c. of a 10% sodium hydroxide solution added. The standard solution must be made up at the same time, as follows: To 20 c.c. of standard creatinin (made by dissolving 15 mgms. of pure creatinin in, and making the volume up to, 1 liter with saturated picric acid) add 1 c.c. of a 10% solution of sodium hydroxide (this should be added at the same time the 0.4 c.c. is added to the unknown). The unknown and the standard solutions are compared after standing for 10 minutes. The formula for computation of this result is as follows: $89 - \text{reading} \times 0.0179 \times 20 =$ mgms. of creatinin plus creatin.

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[95] The standard solution of creatinin is made by dissolving 15 mgms. of pure creatinin in 100 c.c. of a saturated solution of picric acid.

Slightly less accurate results may be obtained by using *0.25 N* potassium bichromate as a standard.

If the accurate value of creatin is desired this is obtained by subtracting the value of creatinin previously determined from the creatin plus creatinin and multiplying the difference by 1.16.

Example.—Let us assume that the reading in the specimen already examined for creatinin (see page 548) was 69. Then $89 - 69 = 20 \times 0.0179 = 0.358 \times 20 = 7.16$ mgms. of creatinin + creatin $= 7.16 - 2.2375$ (*e.g.*, mgms. creatinin) $= 4.9225 \times 1.16 = 5.7101$ mgms. creatin per 100 c.c. blood.

Glucose in the Blood.—The blood of a normal person contains from 0.06 to 0.11% (mean, 0.10%) of glucose. Strouse,[96] using a modified Kowarsky method, found that the normal blood sugar varied from 0.04 to 0.12%, average 0.084%. The figures vary much in the same individual and depend on his diet. The curve has minima before meals and maxima 1 hour after those meals which contain carbohydrates. Above 0.12% is considered a hyperglycemia if the blood was drawn at least 14 hours after the last meal. After a meal it normally may rise to 0.17%. This increase begins at once (within half an hour) after the ingestion of glucose and a little later after a meal rich in starch. In 2 or 3 hours after such a meal the blood-sugar may fall below normal.

In certain pathological conditions the percentage of the glucose of the blood is increased. Among these are diabetes mellitus, nephritis and hyperthroidism especially; some claim also, apoplexy, pneumonia, typhoid fever, tuberculosis, sometimes in cancer, after operations, after prolonged ether anesthesia and in arteriosclerosis. Attention should at this point be called to the importance of changes in the blood volume as explaining a rise or fall in the percentage of glucose. Epstein would limit the term hyperglycemia to an increase in the total amount of glucose in the blood irrespective of its percentage.

Hypoglycemia would seem to be the result of subnormal endocrine functions since it has been noted in myxedema, cretinism, Addison's disease, pituitary disease and also in muscular dystrophy.

In 12 cases of these conditions reported (Myers) the blood-sugar ranged from 0.064 to 0.086%.

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[96] Johns Hopkins Hosp. Bull., June, 1915, vol. xxvi, p. 211.

The question of a "renal threshold" above which glucose will pass over into the urine has attracted considerable attention. Various authors have placed this at from 0.149 to 0.18% but this varies much and even in the same individual may be raised or lowered by various drugs and more especially by conditions producing a diuresis which would tend to produce also a glycuresis. In diabetes mellitus, especially in the cases of long standing and particularly in those complicated by nephritis, there may be a hyperglycemia of from 0.25 to 0.35% or more without glycosuria. This threshold is changed also by exercise. One of Joslin's patients showed that a liberal carbohydrate meal at noon would not produce a glycosuria if he exercised strenuously immediately thereafter.

It is in diabetes mellitus that the amount of glucose in the blood has value in diagnosis, prognosis and treatment. In untreated cases of this disease the percentage usually lies between 0.20 and 0.40% but in well treated cases it may lie within normal limits. After a meal rich in carbohydrates the increase in the blood-sugar is slower than normal, reaching its maximum in 2 hours, while the decline occupies 8 or 10 hours. In cases before death with coma it may be above 0.40%, in cases with nephritis even 0.80% and in 1 such case of Joslin's 12 hours before death it was 1.37% (probably the highest figure on record).

We take the liberty to draw the following conclusions from Joslin's splendid work: First, the younger the patient the lower is the blood sugar apt to be (but the converse is not true). Second, the duration of the disease bears no necessary relation to the amount of sugar in the blood. (Therefore a case does not necessarily become more severe the longer it lasts.) Third, the presence of glucose in the urine is almost invariably preceded 24 hours before by an increase of the sugar in the blood. (On the other hand there may be a hyperglycemia of even 0.50% and no glycosuria.) Fourth, there is an intimate relation between blood sugar and the carbohydrate balance. This depends on the diet but also on the quantity of stored carbohydrate. Fifth, some very mild diabetics, especially those of the hereditary type, have early a low blood sugar per cent., even 0.11%, but a higher one later. Sixth, there is, in cases of long duration, a definite relation between the blood sugar and the patient's assimilation limit for glucose. The lower the tolerance for carbohydrates the higher the percentage of blood sugar and when the tolerance is distinctly high the blood sugar may be little above normal. There are, however, many exceptions to this rule. Seventh, in cases of long duration the blood sugar is seldom high no matter whether the patient's tolerance for carbohydrate is low or high. Eighth, those cases at first clinically severe but which become milder on treatment show corresponding changes in the blood sugar, but those cases which at first appear severe but later prove to be mild could not at first have been recognized as mild by blood analysis. Ninth, treatment is as a rule accompanied by a diminution in the blood sugar, although the patient's urine usually becomes sugar-free before his blood sugar shows any particular diminution. This means that rigorous dietetic treatment should be continued for a long period after the urine is sugar-free. That is, the success of treatment depends on keeping the blood sugar within normal limits as well as on keeping the patient's urine sugar-free.

Renal diabetes on the other hand is a glycuresis due to a lowering of the renal threshold point below the level of the normal blood sugar. Unlike diabetes mellitus renal diabetes is the result of kidney disease. The renal signs are sometimes, but not always, those of nephritis. The condition would seem somewhat analogous to phlorizin glycosuria but resembles more uranium nephritis. Myers suggests that this condition may explain some cases of mild glycosuria which have not the classic symptom of diabetes mellitus. The blood sugar in Myers' cases ranged from .09 to .19% and the sugar in the urine from 0.3 to 1.3%.

Determination of Carbohydrate Tolerance.—Killian's method (quoted from Myers) of determining the carbohydrate tolerance, which now promises to replace Naunyn's test for the assimilation limit (see page 163) since the variable factor of the threshold point of renal excretion is eliminated, is as follows: The patient is given in the morning a standard breakfast consisting of 2 slices of bread, 1 egg in any form and 1 cup of water. Two hours later he empties his bladder and then drinks 200 c.c. of water. One hour later a specimen of blood and 1 of urine are taken as controls. The patient then ingests glucose in the form of a 50% solution, 1.75 gms. (of glucose) per 1 kg. of body weight. The blood sugar is then determined each hour for 3 or 4 hours and its curve plotted. The urine for the 24 hours following the meal is collected and the presence of glucose determined. In one modification of the Goetch test for hyperthyroidism the determination of blood glucose is made. The patient is given 100 gms. of glucose by mouth and then 8 minims of adrenalin are injected under the skin and the blood sugar followed each hour. In a positive test there will be a definite rise of the blood sugar and sometimes a glycosuria.

Case No. 10155, a girl 17 years of age with hyperthyroidism, showed during the Goetch test a rise in systolic blood pressure from 115 to 142 mm. Hg. in about 11 minutes. It returned to 118 in approximately 1 hour. The pulse rose from 100 to 120 per minute. The subjective symptoms were very marked; tremor, which was marked and generalized, and a numb feeling which lasted about 50 minutes. The blood sugar on the fasting stomach was 0.12%. One hour after the ingestion of 100 gms. of glucose by mouth this was 0.19% and at the end of about 5 hours, 0.18%. Hourly urine specimens were sugar-free. The blood creatinin was 2.27 mgms. per 100 c.c. and basalmetabolism 45.7 cal. per square M. per hour.

In case No. 9998 the Goetch test showed a rise in systolic pressure from 130 to 138 mm. Hg. where it remained for 2 hours. The pulse-rate increased from 110 to 128 per minute. The blood sugar rose from 0.17% to 0.20% after 100 gms. of glucose by mouth. In 2½ hours it was 0.18% and in 4½ hours it had fallen to 0.13%. All urine voidings were negative for sugar. This case had a negative Goetch cutaneous reaction.

All the clinical signs of hyperthyroidism were present. The basalmetabolism test gave 48.4 cal. per kgm. per 24 hours, or an increase of 87%.

Case No. 9782, of adenoma of the thyroid with toxic symptoms, gave a positive Goetch test. The blood-pressure rose from 112 to 128 mm. Hg. in about 35 minutes and returned to normal in 1 hour. The pulse rose from 102 to 120 per minute. There were no subjective symptoms. The Goetch cutaneous was negative. But the blood sugar, which before the test was 0.16%, rose in 1 hour after the ingestion of 100 gms. of glucose by mouth to 0.21%. The 2d hour it dropped to 0.17%, the 4th hour it read practically the same and at the 6th hour it read 0.16%. The urine passed the second hour, when the blood sugar was the highest, was negative for sugar but glucose was present in the next 2 hourly specimens. The later specimens were negative. The blood creatinin was 1.7 mgms. per 100 c.c.

Quantitative Determination of Glucose in the Blood.—(Lewis-Benedict method modified by Gradwohl and Blaivas). The blood should be taken in the post absorptive stage, *i.e.*, 14 hours after the last meal, which usually is between 7 and 9 in the morning. The estimation of the glucose of the blood should be begun as soon as possible after the blood is drawn, otherwise the specimen will deteriorate rapidly. One measures 3 c.c. of the filtrate (see page 547) into a sugar tube, a graduated test tube on which are marked 1, 4, 10, 15, and 20 ccs., adds 1 c.c. of saturated sodium carbonate (prepared by dissolving 220 gms. of anhydrous sodium carbonate in 1000 c.c. of distilled water) and mixes it well. The test tube containing this mixture is then immersed in a large beaker of water which is then boiled over a free flame for about 15 minutes and then allowed to cool. This cooled solution is then so diluted with distilled water that it will be weaker in color than the standard picramic acid solution with which it is to be compared in the colorimeter. To this end it is diluted to 10, 15, or 20 c.c. (see marks upon the graduated sugar tube. In average normal cases a dilution to 10 c.c. will suffice but in cases of hyperglycemia it is often necessary to dilute to 15 c.c. or even to 20 c.c. It is now compared in the colorimeter with a wedge of standard picramic acid. The readings should be made as rapidly as possible since the color will soon change.

The standard picramic acid solution is a staple solution and is prepared as follows: Dissolve 0.1 gm. of picramic acid and 0.2 gm. anhydrous sodium carbonate in 30 c.c. warm distilled water and dilute to 1 liter.

Example.—If the dilution was to 10, multiply the difference between the reading and 100 by 0.002; if to 15, by 0.003; if to 20, by 0.004; while if the dilution is to 25, multiply by 0.005; etc.

Identical results may be obtained by using the data presented in Table VI, providing the estimation was made on the basis of a dilution of 10. If it was diluted to 15 c.c., multiply the result by 1.5; to 20 c.c., multiply by 2; etc.

Creatinin will be estimated by this method as glucose but the error is not great since creatinin very seldom exceeds 20 mg. per 100 c.c. of blood. To lessen this error Myers proposes the following method in which a lower dilution of blood is used.

TABLE VI*

ESTIMATION OF BLOOD SUGAR WITH HELLIGE COLORIMETER

Colorimetric reading	Blood sugar in per cent	Colorimetric reading	Blood sugar in per cent	Colorimetric reading	Blood sugar in per cent.
25	0.150	45	0.110	65	0.070
26	0.148	46	0.108	66	0.068
27	0.146	47	0.106	67	0.066
28	0.144	48	0.104	68	0.064
29	0.142	49	0.102	69	0.062
30	0.140	50	0.100	70	0.060
31	0.138	51	0.098	71	0.058
32	0.136	52	0.096	72	0.056
33	0.134	53	0.094	73	0.054
34	0.132	54	0.092	74	0.052
35	0.130	55	0.090	75	0.050
36	0.128	56	0.088	76	0.048
37	0.126	57	0.086	77	0.046
38	0.124	58	0.084	78	0.044
39	0.122	59	0.082	79	0.042
40	0.120	60	0.080	80	0.040
41	0.118	61	0.078	81	0.038
42	0.116	62	0.076	82	0.036
43	0.114	63	0.074	83	0.034
44	0.112	64	0.072	84	0.032

* Myers and Fine. Table copied from Gradwohl and Blaivas.

MYERS AND BAILEY'S METHOD.—To 8 c.c. of distilled water in a 20 c.c. cylindrical centrifuge tube are added 2 c.c. of well mixed oxalated blood (or oxalated plasma). This is then stirred with a glass rod until the blood is well hemolyzed after which about 0.5 gm. of dry picric acid (sufficient to precipitate completely the proteins and to make a saturated solution) is added.

The mixture is thoroughly stirred at intervals of several minutes until it is uniformly yellow, it is then centrifugalized and the supernatant fluid filtered into a dry test tube through a small 4 cm. filter paper.

Three cubic centimeters of the filtrate are measured with a pipette into a tall narrow sugar tube (12×200 mm.) graduated to 3, 4, 10, 15, and 20 c.c. Then 1 c.c. of saturated (22%) sodium carbonate is added and the tube heated in a beaker of boiling water for 15 or 20 minutes. Simultaneously 3 c.c. of a 0.020% solution of glucose in saturated picric acid (this keeps permanently) is treated with a similar amount of sodium carbonate in a sugar tube and heated for the same time as and with the unknown. This serves as the standard. The yellow sodium picrate is converted by the heat and alkali to reddish brown sodium picramate in proportion to the amount of sugar present. The solutions are now cooled to room temperature either by allowing the tubes to stand or by placing them in a beaker of water. The solution in the standard tube is made up to exactly 10 c.c. with water and the contents of the other tube then diluted to some definite volume (as 10, 15 or 20 c.c.) the color of which approximates the color of the standard. It is best to allow a little time to elapse before color comparisons are made. These fluids are then compared in a colorimeter. The standard may conveniently be set at the 15 mm. mark.

Since the 3 c.c. of filtrate is the equivalent of 0.6 c.c. of blood and the 3 c.c. of standard solution contains 0.6 mgm. of glucose, the proportions are the same as if 100 c.c. of blood and 0.1 gm. of glucose had been employed.

$$\frac{S \times D \times 0.1}{R \times 10} = \text{per cent. of blood sugar.}$$

S = depth of standard (15 mm.).
D = dilution of unknown.
0.1 = strength of standard in grams calculated on the basis of 100 c.c. of blood.
R = reading of unknown.
10 = dilution of standard.

To test the purity of the picric acid Folin and Doisy recommend the following method.[97] To 20 c.c. of a saturated solution of picric acid add 1 c.c. of 10% NaOH and allow it to stand for 15 minutes. The color of the alkaline picric solution thus obtained should not be more than twice as deep (measured in a colorimeter) as the color of the saturated acid solution.

For the determination of *acetone*, *diacetic acid* and *hydroxybutyric acid* in the blood see page 197.

Diastatic Activity of the Blood.—The diastatic activity of the blood is increased in conditions of hyperglycemia and therefore in diabetes and nephritis, with the exception of the cases of diabetes due to lues. The increase of diastase in the blood of nephritics is probably due to a decreased elimination of this in the urine. The figures for diastase in normal individuals is given by Myers [98] as 16 and 17%. In diabetes it may range from 24 to 74% running in general parallel to the blood sugar. Myers and Killian [99] suggested that the increase of blood diastase may be an important factor in the production of a hyperglycemia.

DETERMINATION OF THE DIASTATIC ACTIVITY OF THE BLOOD.—Two 2 c.c. samples of oxalated blood, 1 for control, are measured into two 20 c.c. cylindrical centrifuge tubes. The contents of the control is made up to 10 c.c. with distilled water and the other tube to 9 c.c. Both tubes are now placed in a water bath the temperature of which is maintained constantly at 40° C. As soon as the contents of the tubes has been brought to this temperature 1 c.c. of 1% soluble starch (which does not contain over 6% of reducing sugar; this reagent is tested before it is used) is added to the second tube, the contents mixed and the tubes incubated at 40° C. for exactly 15 minutes. At the end of this time about 0.5 gm. of dry picric acid is at once added to each tube and the mixtures stirred. When the proteids have been precipitated the tubes are centrifugalized and the yellow supernatant fluids filtered. The sugar in 3 c.c. portions of each of the filtrates is now estimated by the method described on page 552. Correction is made for the sugar originally present in the blood (with the aid of the control) and for the reducing bodies of the soluble starch if any existed.

The results are recorded in terms of the percentage of the soluble starch (10 mgms.) transformed to reducing sugars (calculated as glucose) by the 2 c.c. of blood.

$$\frac{S \times D \times 2.0}{R \times 10} = \text{mgms. of reducing sugar in terms of glucose for 2 c.c. of blood.}$$

S = depth of standard (15 mm.). D = dilution of unknown in cubic centimeters. 2.0 = strength of standard in milligrams compared to 2 c.c. of blood. R = reading of unknown in millimeters. 10 = dilution of the standard.

The difference between the results of the control and the test specimens multiplied by 10 (10 mgms. of the soluble starch were used) gives the percentage transformation of the starch to reducing sugar provided the soluble starch requires no correction. For example, a diabetic blood with 0.26% blood sugar would give a control of 5.2 mgms. (since 2 c.c. were used). Suppose that the specimen gave 9.8 mgms. reducing sugar and that the soluble starch contained 6% reducing substance, $9.8-(5.2+0.6)\times 10=40$, the diastatic activity.

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[97] Jour. Biol. Chem., 1916–17, xxviii, p. 349.

[98] The Jour. of Lab. and Clin. Med., July, 1920, vol. v, No. 10, p. 640.

[99] Jour. Biol. Chem., 1917, vol. xxix, p. 179.

Blood Lipoids. [100]—Joslin, quoting Bloor, classifies the lipoids in the blood as: (1) Glycerides of the fatty acids, especially of oleic, stearic and palmitic acids. (2) Lecithin-like bodies, which are compounds of glycerin, fatty acid, phosphoric acid and cholin and called often "phosphatides." (3) Cholesterol, a stable secondary alcohol belonging to the terpene series and containing one double bond. (4) Cholesterol esters, which are combinations of cholesterol and a fatty acid.

The lipoids may be classified also by grouping the constituents of the above bodies, as: (1) Total fatty acids, which vary in the whole blood between 0.29 and 0.42%; (2) lecithin, which varies from 0.28 to 0.33%; and (3) cholesterol which varies from 0.19 to 0.25%. Cholesterol in all cases runs parallel to the fatty acids, therefore its determination should give valuable information regarding the lipoid content of the blood, but not as valuable as that of the total fatty acids, since the lowest figures of the latter in the diabetic blood were at the upper limits of normal, while those for cholesterol overlapped the normal range.

We copy from Joslin the table (for which he gives Bloor credit) of lipoids of normal blood.

LIPOIDS OF NORMAL BLOOD *

	Total fatty acids, grams per 100 c.c.			Lecithin, grams per 100 c.c.			Cholesterol, grams per 100 c.c.		
	Whole blood	Plasma	Corpuscles	Whole blood	Plasma	Corpuscles	Whole blood	Plasma	Corpuscles
Per cent. variation of high above average...	14.0	20.0	32.0	10.0	24.0	14.0	12.0	35.0	20.0
Highest normal........	0.42	0.47	0.45	0.33	0.26	0.48	0.25	0.31	0.24
Average (19) † normals.	0.37	0.39	0.34	0.30	0.21	0.42	0.22	0.23	0.20
Lowest normal........	0.29	0.30	0.27	0.28	0.17	0.35	0.19	0.19	0.17
Per cent. variation of low below average...	22.0	23.0	21.0	7.0	19.0	17.0	14.0	17.0	15.0

* The results of the analyses of blood lipoids of both males and females have been combined in this table.

† The number of analyses are given in parentheses.

The influence of food on the blood fat is striking. Fasting and narcosis cause an increase in the blood lipoids (probably only if the animal was previously well fed).

The total fatty acids of the blood have been found increased in nephritis, pneumonia, pregnancy and in the experimental anemias of animals.

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[100] Joslin, Treatment of Diabetes Mellitus, Second Edition, 1917, page 96.

Lecithin appears to be an intermediate stage in the metabolism of fat, formed in the red corpuscles from the fat absorbed from the plasma. Lecithin has been found increased in nephritis and in leukemia (in the corpuscles).

Cholesterol (cholesterin) does not seem to be increased by the mere ingestion of fat, but is increased in narcosis, alcoholism, pregnancy, jaundice and nephritis and is decreased in cachexia. Schmidt[101] found in normal persons the following figures:

CHOLESTERIN IN NORMAL BLOOD-SERA

Age	Observations	Amount in grams per liter
10–19	1	1.40
20–29	5	1.60–1.27–1.75–1.45–1.50
30–39	3	1.65–1.25–1.35
40–49	1	1.20
50–59	3	1.45–1.55–1.20
60–69	4	1.30–1.20–1.30–1.35

For a series of very careful and interesting studies on cholesterol the reader is referred to those of Luden.[102]

By lipemia is meant an increase of the visible fat of the blood plasma, yet the condition is more one of lipoidemia since it is due to an increase of the glycerides of the fatty acids, of lecithin and cholesterol. A lipemia is physiological in sucklings, in very obese persons, in some pregnant women and in adults after a heavy meal.

Recently, however, the term has been reserved for those cases in which the plasma is milky 14 hours after the last meal. Severe diabetes mellitus is the only disease in which lipemia is frequent enough to be of special significance (see page 452). Futcher[103] has contributed an article on this subject. Fraser[104] reported a case with 16.44% of fat in the blood and 18.94% in the pleural exudate. The record case is Fischer's, with 18.129% in the blood.

In diabetes mellitus the blood lipoids, chiefly of the plasma, were increased 50% in 26 of 28 cases. A true lipemia, or milky plasma, due to the presence of an abundance of fatty globules which in extreme cases may even rise as a cream, may easily be demonstrated soon after a rich meal in a severe case, but is rare in cases under treatment if the blood is drawn 14 hours after the last meal.

The amounts obtained by chemical analysis are shown in the following table copied from Joslin.

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[101] Arch. of Int. Med., Jan., 1914, vol. xiii, p. 121.
[102] Jour. Lab. and Clin. Med., 1916, vol. i, p. 662; 1917, vol. iii, p. 93.
[103] Jour. of Am. Med. Assoc., Oct. 21, 1899.
[104] Scot. Med. and Surg. Jour., 1903, p. 200.

COMPARISON OF BLOOD LIPOIDS OF NORMAL AND TWENTY-EIGHT DIABETIC INDIVIDUALS*

	Total fatty acids, gms. in 100 c.c.			Lecithin, gms. in 100 c.c.			Cholesterol, gms. in 100 c.c.		
	Whole blood	Plasma	Corpuscles	Whole blood	Plasma	Corpuscles	Whole blood	Plasma	Corpuscles
Diabetic extremes..	.41–.76	.46–.93	.33–.62	.26–.50	.17–.48	.32–.60	.19–.44	.16–.65	.17–.24
Diabetic average (34)	*.52*	*.59*	*.43*	*.36*	*.30*	*.46*	*.29*	*.36*	*.20*
Normal average (19)	*.37*	*.39*	*.34*	*.30*	*.21*	*.42*	*.22*	*.23*	*.20*
Normal extremes...	.29–.42	.30–.47	.27–.45	.28–.33	.17–.26	.35–.48	.19–.25	.19–.31	.17–.24

*Compiled by Joslin from tables of W. R. Bloor, Jour. Biol. Chem., 1916, xxvi, p. 424

Joslin concludes that the more severe the diabetic condition the more abnormal the quantities of lipoids in the blood. While in general the relations between the various lipoids in diabetes are practically the same as in normal individuals yet there is a tendency for the total fatty acids to increase out of proportion to the other constituents. The best explanation for this increase in the blood lipoids is a disturbance of the fat-burning mechanism and the consequent accumulation of metabolites.

The presence of visible fat in lipemia, seen so often in the patients on the diets formerly so popular but so seldom in patients under Allen's treatment, would seem to depend directly on the abundant fat of the diet which contained as little as possible of carbohydrates.

The fat will remain in solution until the lecithin-forming function fails.

The lipemia sometimes present in blood in which there is not a sufficient increase of the blood lipoids to warrant its presence is explained by changes which take place in a clear diabetic plasma after it has stood more than a few hours.

Joslin was unable from a study of his cases to demonstrate any definite relation between blood lipoids and acidosis, the duration of the disease, the prognosis or the amount of blood sugar.

QUANTITATIVE DETERMINATION OF THE LIPOIDS OF THE BLOOD.—(Joslin, p. 207.) The blood should be obtained 14 hours (from 8 to 16) after the last meal and the analysis begun as soon as possible (not later than 2 hours) after the blood is drawn.

Three cubic centimeters of freshly drawn and well mixed blood are run in a slow stream of drops into a graduated flask containing about 80 c.c. of a mixture of 3 parts alcohol and 1 part ether (both redistilled), which is kept in constant motion by rotating the flask. The solution is raised to boiling by immersing the flask in a water bath (with frequent shaking to prevent superheating), then cooled to room temperature, made up to volume with the alcohol-ether mixture, mixed and filtered. This extract in tightly stoppered bottles in the dark will keep unchanged for several months.

From 5 to 20 c.c. (ordinarily 10 c.c.) of the extract, *i.e.*, that amount which would contain about 2 mgms. of "fat," are measured with a pipette into a small beaker and saponified by evaporation just to dryness with 2 c.c. of $1N$ sodium ethylate (made by dissolving cleaned metallic sodium in absolute alcohol). After evaporation is complete 5 c.c. of alcohol-ether are added and the mixture heated slowly to boiling.

A similar solution is prepared by measuring 5 c.c. of the standard fat solution (see below) into a beaker and heating to boiling as above. Fifty cubic centimeters of distilled water are now added to each beaker and the solutions mixed by stirring, taking care that all the material in the saponification beaker is dissolved. To standard and test solutions are added, as nearly simultaneously as possible, 10 c.c. of dilute (1 to 4) hydrochloric acid and the solutions allowed to stand 5 minutes after which they are transferred to the comparison tubes of the nephelometer. If bubbles appear on the walls of the tubes they should be removed by inverting the tubes 2 or 3 times. The movable jacket of the standard tube is set at a convenient point, generally 50 mm. (Richard's nephelometer), and comparisons made by adjusting the jacket on the test solution until the images of the 2 solutions show equal illumination. Not less than 5 readings are made, alternately from above and below, and the average taken as the correct reading.

The standard solution used is an alcohol-ether solution of pure triolein of which 5 c.c. contain about 2 mgms. of fat. Freshly redistilled absolute alcohol and pure dry ether should be used for making the standard solution.

Cholesterol.—For the determination of the cholesterol of the blood 2 c.c. of the whole blood, plasma, or serum are run in a slow stream of drops from a pipette into a 100 c.c. graduated flask containing about 75 c.c. of a mixture of redistilled alcohol, 3 parts, and ether, 1 part. The contents of the flask should be kept in motion during the process so that there can be no clumping of the precipitated material. The contents of the flask is now heated to boiling by immersion in a water-bath (with constant shaking to avoid super-heating), cooled to room temperature, filled to the 100 c.c. mark with the alcohol-ether mixture, mixed and filtered. This filtered liquid will keep tightly in a stoppered bottle in the dark unchanged for a considerable time in case it is not convenient to complete the determination at once.

The slow addition of blood to a large quantity of alcohol-ether will precipitate the protein material in finely divided form and so brief heating is adequate for the complete extraction of serum or plasma. The extraction is not so complete in the case of whole blood and yet whole blood is to be preferred because of the higher values obtained.

One now measures 10 c.c. of the alcohol-ether extract into a small flat-bottomed beaker and evaporates *just* to dryness over a water-bath or electric stove. Overheating would produce a brownish color which would pass into the chloroform and render the subsequent determination difficult or impossible. The cholesterol is extracted from the dry residue by boiling it out 3 or 4 times with successive small portions, 3 c.c., of chloroform each of which is allowed to boil down to half its volume or less and then decanting into a 10 c.c. glass-stoppered graduated cylinder. The combined extracts after cooling (5 c.c. or less) are then made up to 5 c.c. The solution should be colorless but not necessarily clear, since a slight turbidity will disappear on adding the reagents.

To this solution are added 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid, the fluids mixed and placed in the dark for 10 minutes to allow for the development of the color. The specimen is then compared in the colorimeter (Hellige) with a standard cholesterol solution which one has been making while preparing the specimen in order that the colors of the unknown and the standard solutions may develop at the same time since the colors fade rather rapidly. It is very important that the wedge and the cup of the colorimeter be perfectly dry.

For the preparation of the standard cholesterol solution 2 c.c. of an 0.08% freshly prepared chloroform solution of cholesterol is pipetted into a dry, accurately graduated 25 c.c. cylinder and made up to 10 c.c. with chloroform. One then adds 4 c.c. of acetic anhydride and 0.2 c.c. of concentrated sulphuric acid.

An aqueous solution of Naphthol Green B can also be used as a standard. The cholesterol content of 0.2 c.c. of blood, serum, or plasma, can be obtained from Table VII. This table is suitable for both standards (pure cholesterol or Naphthol Green B). The result multiplied by 500 will give the percentage of cholesterol.

TABLE VII*

ESTIMATION OF CHOLESTEROL WITH THE HELLIGE COLORIMETER

Colorimetric reading	Cholesterol mgms. dilution of 5 c.c.	Colorimetric reading	Cholesterol mgms. dilution of 5 c.c.	Colorimetric reading	Cholesterol mgms. dilution of 5 c.c.
15	0.74	35	0.57	55	0.40
16	0.73	36	0.56	56	0.40
17	0.72	37	0.55	57	0.39
18	0.71	38	0.55	58	0.38
19	0.70	39	0.54	59	0.37
20	0.69	40	0.53	60	0.36
21	0.69	41	0.52	61	0.35
22	0.68	42	0.51	62	0.35
23	0.67	43	0.50	63	0.34
24	0.66	44	0.50	64	0.33
25	0.65	45	0.49	65	0.32
26	0.65	46	0.48	66	0.31
27	0.64	47	0.47	67	0.30
28	0.63	48	0.46	68	0.30
29	0.62	49	0.45	69	0.29
30	0.61	50	0.45	70	0.28
31	0.60	51	0.44	71	0.27
32	0.59	52	0.43	72	0.26
33	0.59	53	0.42	73	0.25
34	0.58	54	0.41	74	0.24

* Myers and Fine, Table copied from Gradwohl and Blaivas.

Suppose the reading is 60. This would equal 0.36 mgm. of cholesterol in 0.2 c.c. of blood, plasma, or serum. Then 0.36 × 500 = 180 mgms. or 0.18%.

For the preparation of Naphthol Green B, dilute 2 c.c. of a 0.1% aqueous solution of the dye to 17 c.c. with distilled water. The diluted solution appears to keep for a little time, while the concentrated solution apparently will keep for a considerable time. The permanency of the solution and the fact that the color is practically identical with that obtained from cholesterol makes the standard very convenient. Myers and Fine advise, however, to restandardize each new solution.

The method which Schmidt (*q.v.*) used, which was modified from Grigaut, and Weston and Kent, is as follows:

> Two cubic centimeters of blood-serum were placed in a pressure bottle of 150 c.c. capacity. To this was added 20 c.c. of 1% solution of potassium hydroxide dissolved in 50% alcohol. The cover was screwed down and the whole placed in a boiling water-bath for from 15 to 20 minutes. After removal from the water-bath, the contents of the pressure bottle were allowed to cool and were then shaken with 50 c.c. of ether. The ether was decanted into a separating funnel and 30 c.c. more of ether was added to the remaining fluid and shaken vigorously as before. This was again decanted into the funnel and any aqueous liquid which separated from the ether removed. The ether was then washed with 80 c.c. of distilled water. The ethereal extract, after separation from the wash water, was poured into an evaporating dish and evaporated nearly to dryness, leaving behind the small yellow oily droplets containing the cholesterin. Five cubic centimeters of chloroform (Merck's blue label) was then placed on the evaporating dish and rotated carefully so as to dissolve the oily droplets as completely as possible. This chloroform solution was transferred to a convenient receptacle, and a second 5 c.c. of chloroform was then used to wash the evaporating dish and were added to the first 5 c.c.

The amount of cholesterin in the chloroform solution was determined by comparing its color reaction with those of cholesterin chloroform solutions of known strength. These standard solutions were prepared by dissolving Merck's cholesterin in pure chloroform (Merck's blue label) and by preparing a series of dilutions of varying strength so that the amount of cholesterin in 1 c.c. of chloroform varied from 0.0002 to 0.0004 gm. A set of test-tubes of uniform bore were arranged in a rack and 1 c.c. each of these known solutions of cholesterin were added in ascending series, as was also 1 c.c. of the cholesterin solution prepared from the blood-serum. It was often necessary to dilute the latter so that the amount would fall within the scale of the known quantities. To each tube was now added 0.1 c.c. of concentrated sulphuric acid, and the mixture was thoroughly shaken. After 30 minutes 1 c.c. of chloroform was added to each tube and after being placed for 15 minutes in a dark room, the color of the unknown solution was compared with that of the known. This method was found to be exceedingly sensitive and differences in the original fluid of 0.00005 gm. per c.c. could be detected. Successive readings from the same serum also gave concordant results, and even after several days' standing little or no diminution in the amount of the cholesterin in the serum could be discovered.

Quantitative Determination of Fat in the Blood.—This method is valuable when large amounts of blood can be obtained. The method chosen by Bonninger, in Salkowski's laboratory[105] is as follows: From 5 to 30 gms. of blood are mixed with 10 to 20 volumes of 96% alcohol, the precipitate ground fine and then allowed to stand one or two days. It is then filtered, the precipitate extracted several times with alcohol in the same way, then twice with from 5 to 10 volumes of ether, digesting it each time for 1 day. All these extracts are then combined, evaporated, the residue repeatedly taken up in absolute alcohol, and this evaporated, then filtered, dried, and weighed. Extracting twice with alcohol alone would give 96% of the total fat.

BACTERIOLOGY OF THE BLOOD

Assuming on the part of the worker a thorough training in bacteriological technic, we shall consider below only such special points as may be useful in the study of the blood.

The success of blood-cultures is in part dependent upon the obtaining of a sufficient quantity of blood for observation, 15 or 20 c.c. being the usual amount withdrawn, and on its quick dilution in a large volume of physiological salt solution or other dilute medium in order to protect the organisms from the bactericidal properties of the blood which increase after it is drawn. In general, it is obtained from the median basilic or cephalic vein although a smaller vein on the dorsum of the hand or foot may be used. Incision of the skin to expose the vein is not necessary unless the patient is very fat or edematous. For typhoid cultures in bile mediums where only a small amount of blood is needed the finger-tip or the lobe of the ear may be cleansed with soap, then alcohol and ether, then coated with collodion. The skin is then punctured through the collodion and the blood allowed to drip directly into the tube.

If the skin is carefully cleansed the chance of contamination by skin organisms is negligible. Those very careful scrub the site of operation (puncture) with green soap and hot water, then rub it over with Harrington's solution, wash it with ether and alcohol and then cover it with a wet bichloride (1 in 1000) compress. But more merely paint the skin a deep brown color with the tincture of iodine and then may or may not rub it clean with 95% alcohol. This is quite as satisfactory as the more elaborate method.

If a syringe is to be used it should have a capacity of at least 20 c.c. and a glass barrel which is perfectly true. Instead of the ordinary washer for the needle a piece of soft black rubber tubing may be cut and, after perforating it with a pin, slipped over the nipple. This withstands boiling longer and gives a tighter joint. A fresh rubber should be used for each culture. The steel, or better irido-platinum, needle should be short and stiff, sharp and of moderately large caliber. The syringe, needle and a pair of forceps are sterilized in the autoclave or by boiling them for 15 minutes. The forceps are used in putting the needle on the syringe. The more popular apparatus now is that introduced by Rosenau (Fig. 129) which consists of a milk bottle of 200 c.c. capacity almost filled with the liquid medium and closed with a doubly perforated stopper, the one for the tube carrying the needle, the other for a tube through which suction can be made. By this means contaminations are avoided and the blood will at once mix with the medium. A moderately tight bandage is placed on the arm proximal to the site of operation to distend the vein. The skin may be anesthetized with ethyl chloride spray, but this is seldom necessary. The needle is plunged through the skin directly into the vein and the piston is drawn slowly, allowing the syringe to fill with blood, or suction made until the required amount of blood is obtained. The bandage should be removed before the needle is withdrawn since this will prevent bleeding. After withdrawal, the needle and washer of the syringe are removed and the media inoculated quickly. The tip of the syringe should be passed through the flame of an alcohol lamp before inoculating each tube.

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[105] Zeits. f. klin. Med., 1901, vol. xlii.

If the blood must be sent to a laboratory in order that cultures may be made, it may be discharged from the syringe into tubes or flasks containing an equal volume of sterile isotonic ammonium oxalate (ammonium oxalate 2, sodium chloride 6 and water 1000) solution.

Agar tubes melted and cooled to about 45° C. are used for making plates and bouillon or litmus milk in flasks containing 100 c.c. are preferred for fluid media. The plates should be poured at once. A medium of ox-bile, or of ox-bile and peptone, is now considered best for B. typhosus.

The amount of blood to be poured into each tube or flask varies somewhat according to the type of organisms suspected to be present, from equal parts of blood and agar to one volume of blood in five of agar; in flasks, 1 to 2 c.c. of blood in 100 c.c. of medium.

The colon group grows better in bouillon, the pneumococcus better in milk. Anaerobic cultures may be made in the ordinary ways.

If after 24 hours' incubation the plates show only a few surface colonies, contamination certainly has occurred. Only deep colonies which appear similar in several or all plates should be used for subculture. True mixed infection in the blood is uncommon. Plates and flasks should be examined daily for from 5 to 14 days before discarding them as sterile.

Value of Blood-Cultures for Diagnosis.—With the increase of laboratory facilities blood-cultures have become more and more important in diagnosis. In many instances this affords the only means of accurate diagnosis. Following improvement in methods positive blood-cultures have been more and more frequently obtained. Formerly we were able to grow only the hardier organisms or those organisms in bloods which had lost some of their protective properties. Now certain organisms are so often obtained that their pathological importance is doubted. Especially is this true of the so-called diphtheroid group. A blood-culture which contains these is now considered as "negative" and the same may prove to be true of some varieties of streptococci. In other words, the discovery of an organism in the blood must be evaluated since its presence may or may not be important in the diagnosis of the patient's present condition.

The hardier pyogenic organisms (streptococci and staphylococci) are usually readily obtained in cultures in cases of general infections, osteomyelitis or malignant endocarditis due to their presence. Some idea of the intensity of the infection may be gathered from the number of colonies obtained per cubic centimeter of blood used.

THE STREPTOCOCCUS GROUP.—During the last few years different varieties of streptococci (see page 19) have claimed especial attention. For the study of these we quote Kinsella's technic.

From 30 to 40 c.c. of blood are withdrawn from the cubital vein. From 5 to 10 c.c. of this blood are planted directly in flasks of broth, or dextrose agar plates are poured, using from 2 to 3 c.c. of blood in each. Another 5 c.c. are hemolyzed in 40 c.c. of sterile distilled water and the sediment, after centrifugalization, planted in a deep tube of melted ascitic-dextrose agar and allowed to harden before incubating.

The cultural characteristics of the streptococci include (*a*) their appearance in broth, (*b*) their effect on serum-dextrose-agar, (*c*) their solubility in bile, (*d*) the lethal dose of a 24-hour broth culture for white mice, (*e*) their effect on red blood-cells (this characteristic is tested by planting cultures on blood-agar plates and by mixing a 24 hour broth culture with a 5% saline suspension of sheep red blood corpuscles (Lyall), and (*f*) the fermentation reactions on litmus milk and on raffinose, inulin, salicin and mannite serum water mediums. Each strain studied should be started from a single colony.

For the purpose of attempting a classification of the streptococci on an immunologic basis, rabbits are inoculated with saline emulsions of killed streptococci at 4-day intervals in doses equivalent to 10 c.c. of the broth culture. The injections are continued until the serum of an animal shows marked complement-binding capacity for the corresponding streptococcus antigen. The serum is then tested against the other strains for cross fixation and cross agglutination.

TYPHOID BACILLI have been demonstrated in the blood in upward of 75% of a series of cases by Cole, Buxton, Schotmüller, Hewlett and others, often days or even weeks before the Widal test is positive.

IN THE PARATYPHOID AND PARACOLON INFECTIONS the isolation of the organism from the blood or stools is the only definite means of differentiating these cases from those of true typhoid fever.

In pneumococcus infections the percentage of positive cultures is fairly large, the organism being found principally in the graver cases.

Among other organisms of less frequent occurrence in the blood during life may be mentioned: B. aërogenes capsulatus, B. coli, B. pyocyaneus, B. anthracis, etc.

Blood cultures should be made if possible during a period of rising temperature and yet positive results may be obtained during the hours of apyrexia.

Blood-cultures involve but little inconvenience to the patient and should be repeated many times before the attempt to isolate an organism is abandoned.

AGGLUTINATION PHENOMENA

Through the action on the tissues of certain bacteria soluble bodies appear in the blood known as agglutinins. These agglutinins, when sufficiently concentrated, have the property of clumping and rendering non-motile that organism whose activities gave rise to their production.

The nature of the interactions between the bacteria and the agglutinating serum is unknown and theoretical discussion of the phenomena would carry us too far afield except to say that agglutination would seem due to the earliest changes in that process which ends in bacteriolysis.

Gruber-Widal Test.—This is an agglutination phenomenon applied to the diagnosis of typhoid fever.

Cultures.—A standard stock culture of B. typhosus, and one which is actively motile, should be grown for this purpose. An organism cultivated through many generations on artificial media is preferred. A subculture on agar from this stock culture from 12 to 24 hours old is used in the test. Some authorities prefer fresh (10 to 18 hour old) bouillon cultures from the stock. Others use bouillon cultures killed by the addition of carbolic acid, formalin, etc. Hastings has devised a method, based on Ficker's "Typhus diagnosticum," which yields very satisfactory results, viz.: To a mixture containing aqueous 5% carbolic acid 5 c.c., glycerin 10 c.c., sterile 0.8% sodium chloride solution 85 c.c. are added the growth scraped from two 24-hour agar slant cultures of the typhoid bacillus. Carbolic acid is preferred to formalin for this purpose since the latter may precipitate flocculi of proteid from the serum. The bacilli are gradually and thoroughly rubbed into the solution with a small spatula and this allowed to stand 5 or 6 days before using. The test is made by mixing equal volumes of this suspension and of the diluted sera.

More satisfactory results are obtained with emulsions of the fresh living culture on agar (rather dry slants are best) in 0.8% salt solution or in bouillon. A loopful of the growth is rubbed against the side of the tube of salt solution until it is thoroughly broken up and is then gradually mixed with the fluid. With a little care a suspension free from clumps may be secured. One loopful (using a loop of standard size) in 1 c.c. of salt solution will give a fairly constant suspension for comparative work.

Collecting the Blood.—Glass tubes 2 inches in length and 1/4 inch in diameter are drawn out into a capillary at both ends. (See Fig. 130.)

The blood is allowed to flow from a free-flowing puncture in the ear or finger-tip into the tube by capillary attraction until it is ⅔ full. The tube then lies flat until the serum has separated from the coagulum. It is then filed and broken off at a point just beyond the clot and the serum withdrawn with a capillary pipette. The separation of the serum may be hastened and more obtained by sealing the tips of the tube in a flame and centrifugalizing it for a few minutes. This will condense the clot at one end of the tube. If it is desired to preserve the specimen or to send it away, both its ends may be sealed in the flame or with sealing-wax. Serum is best kept in a sterile tube and separated from the corpuscles. If larger amounts of serum are required, a vein should be aspirated with the syringe (see page 561).

Diluting the Serum.—A simple and very satisfactory method of diluting the serum is as follows: A piece of ¼-inch glass tubing is drawn into a long capillary, as shown in Fig. 131. This is plunged into serum in the collecting tube and the capillary allowed to fill, care being taken not to stir up the corpuscular layer. The serum is dropped from this capillary into the tubes or dishes in which the dilutions are to be made. A small water-color porcelain palette is very convenient for making a number of dilutions, or salt-cellars or watch-crystals may be used. As a routine at least 2 dilutions of each serum should be made, 1 to 50 and 1 to 100.

Using this same pipette, which has been washed out with distilled water to remove every trace of serum and then dried in the flame, one now adds to the first drop of serum 24 drops and to the second 49 drops of 0.8% salt solution. The addition to these dilutions of an equal volume of the suspension of the typhoid culture will give us dilutions of 1/50 and 1/100. In the same way any other desired dilution may be made. If greater accuracy or very high dilutions be desired special mixing pipettes similar to the Zeiss melangeur for blood counting may be employed. Again, using such a melangeur the whole blood may be diluted with salt solution and each 2 volumes of blood counted as 1 volume of serum. This mixture is allowed to settle or, better, is centrifugalized to remove the corpuscles and the diluted serum used for the macroscopic or microscopic tests.

A. Macroscopic Method.—The macroscopic method depends on the agglutination and eventual precipitation of the organisms in clumps visible to the naked eye leaving a clear supernatant fluid. The serum is diluted in a small test-tube and a suspension of the organisms, living or killed, added; or, what is perhaps better, the full dilution of serum with salt solution, 1 in 50 or 1 in 100, is first made and the solid growth of the organisms then suspended in the diluted serum (see page 564). The tube is then examined by strong transmitted light to be sure that its contents are homogeneous and free from accidental clumps. A narrow band of light from a lamp enclosed by a screen aids in detecting the early appearance of clumping. The test is considered positive if at a dilution of 1/50 or higher there is general clumping in 1 hour and complete precipitation leaving a clear supernatant fluid after 24 hours. The reaction is hastened if the tubes are placed in the thermostat.

This method has the advantage of simplicity in that a microscope is not required and that killed cultures may be used, thus obviating the necessity for a thermostat and culture media.

Several pharmaceutical laboratories in this country now sell killed cultures for the macroscopic Widal.

B. THE MICROSCOPIC METHOD.—The diluted serum may be mixed with the requisite volume of the typhoid suspension by the use of pipettes, as above noted, and a drop of the mixture observed on a hanging drop slide. Or, we may mix the 2 on the cover-slip directly. To do this we use a platinum loop of stiff wire, the plane of the loop being at right angles to the handle and the diameter of the loop being constant. The loop is dipped vertically into the serum dilution and the drop so obtained placed on the center of the cover-slip. The loop is flamed off and dipped into the typhoid suspension in the same way and the 2 drops thoroughly mixed on the cover-slip. Approximately equal volumes are readily obtained by this simple method. The cover-slip is then inverted over the well of a hanging drop slide which previously has been ringed about with olive oil or vaseline and the preparation is then ready for examination. The hanging drop is observed with a moderately high dry lens (Zeiss D. or Leitz ⅙ in.) and by artificial illumination. The Argand burner or oil-lamp with a yellow flame is preferred. The light is stopped down with the diaphragm so as to bring out the refractivity of the bacteria.

Inspection of the freshly made hanging drop should show an absence of clumps and all the organisms in active motion (see Fig. 132). After the lapse of 1 hour if the test is positive (see Fig. 133) the organisms in a dilution of 1/50 will be seen to have lost their motility and to be collected entirely in clumps. The presence of a few non-motile free organisms in a field otherwise well clumped is not considered to vitiate the test.

In such a preparation, if serum diluted 1:50 or more will in 1 hour agglutinate all the organisms into clumps, the result is positive; if the clumping is fair and the single organisms are non-motile this result also is considered positive; but if even with fairly good clumping many of the single organisms are still in motion the result is considered merely suggestive.

It is frequently noticed that the clumping is better at the higher dilutions and that there is marked bacteriolysis in dilutions of 1:10 or 1:20 or even higher. It also is true that many normal serums will give perfect agglutinations at 1:10 and show no trace of the reaction in a 1:50 or higher dilution; therefore the tests based on the low dilutions alone are unreliable.

The macroscopic method has rapidly gained favor in the best laboratories and is probably less open to error than the microscopic, provided strict limits of time and dilution (1 hour at dilution of 1 in 50 or higher) are observed. There is so much difference of opinion as to what constitutes microscopic clumping that it is often difficult to compare results. Some authors consider the aggregation of a very few of the organisms to be a positive test. These differences of opinion have led to much confusion, particularly in experimental work.

AGGLUTINATION WITH DRIED BLOOD.—If blood dried on glass, tin-foil, or glazed paper is to be tested the results are fairly accurate if the blood used is carefully weighed and the dilution based on weight instead of volume.

VALUE OF AGGLUTINATION REACTIONS IN TYPHOID FEVER.—While the Widal reaction rarely fails to appear sooner or later in each case of typhoid fever it may be long delayed, even until convalescence, and it is seldom present before the seventh or eighth day, so that it is not an aid in early diagnosis. Still, it remains our most certain confirmatory test after the bacilli have disappeared from the circulating blood and is indispensable in abortive, doubtful, and obscure cases.

The persistence of the agglutinative reaction is variable. In some it remains positive for a few weeks, in others for years, after the attack of fever. These cases of long persistent Widal have been attributed to the presence of typhoid baccilli in the gall-bladder, in gall-stones, or in the urinary bladder.

The agglutination of B. typhosus by normal serum diluted 1/50 in 1 hour is so rare as to be negligible.

The question of "associated" or "group" agglutinations, that is, the agglutination of two or more closely related organisms by the same serum, as B. coli, B. alkaligines and B. typhosus, is too complicated to find place here. Suffice it so say that the limited time and the high dilution employed in our tests are sufficient to give us reliable specific results.

Paracolon Infections.—While the 11 or more types of paratyphoid and paracolon bacilli often give highly specific agglutinations the presence of associated agglutinins should be considered and the diagnosis of any one type of paracolon organism by the agglutination reaction alone would be questionable unless confirmed by positive cultures.

Other Agglutinations.—The agglutination reactions have been applied to many different organisms with more or less definite results, but in most cases they have not reached any considerable diagnostic value and are often very difficult of application.

OPSONINS

Phagocytosis has long been looked upon as one of the strong defenses of the body against infection. It has been observed that as a rule bacteria are ingested by the phagocytes more readily in the presence of serum than in its absence. This action of the serum in facilitating phagocytosis is referred to the presence in the serum of a hypothetical " body " to which the term " opsonin " has been applied and which is supposed to act upon the bacteria, producing some change in them which facilitates their ingestion by the phagocytes.

That the action of the serum is directed toward the bacteria rather than the phagocytes is indicated by the following results: Bacteria which have remained in contact with serum at 37.5° C. for a short time and then repeatedly washed in normal salt solution to remove the serum are readily ingested by washed leucocytes, while bacteria which have not been exposed to the action of serum are ingested to a much less extent by washed leucocytes. This is not universally true of all varieties of bacteria. Some, such as Bacillus pyocyaneus, may be readily ingested by the leucocytes without previously having been acted on by serum and a greater or less amount of phagocytosis of practically any variety of bacterium occurs independently of the action of serum (the so-called "spontaneous" phagocytosis). Spontaneous phagocytosis is said to be inhibited by a 1.2% concentration of sodium chloride. Not only do different varieties of bacteria differ in their resistance to phagocytosis but different strains of the same variety may show a marked variation in their resistance to phagocytosis even under the influence of the same serum. In general the more virulent the strain the more resistant it is to phagocytosis.

Opsonins are placed in the category of immune bodies along with agglutinins, precipitins, bacteriolysins, etc., and like them are regarded as specific bodies; that is, just as the agglutinins for different varieties of bacteria are specific so there are specific opsonins. Like the agglutinins also, opsonins occur normally in the serum and in greater amounts as a result of infection or immunization. The former are designated as "normal" opsonins, the latter as "immune" opsonins. Normal opsonins are said to be thermolabile, being destroyed by an exposure to 57° C. for ½ hour, while immune opsonins resist this exposure and are therefore thermo-stabile.

If the foregoing observations are correct and if opsonins are really specific immune bodies playing a very important part in the defensive mechanism of the body, any method which would enable their accurate estimation might be of great service in diagnosis and prognosis and any means of regulating their presence in the body might be of great therapeutic importance.

For diagnostic purposes the estimation of any of the other immune bodies—agglutinins, precipitins, complement-fixing amboceptors, etc.—is at present more valuable than the opsonic index. Prognostically little value can be attached to any of them and by therapeutic measures these other bodies can be influenced more than can the opsonins.

The power of the serum in favoring phagocytosis is spoken of as its opsonic power. The opsonic index of a given individual is the ratio of the opsonic power of his serum to the opsonic power of the serum of a normal individual.

Since the opsonic index is so little used now, although the reason may be our faulty methods of technic, we will not give a detailed description of the technic, merely enough that the student may have a general idea of a test so often referred to in literature.

Equal quantities of the patient's serum, of a suspension of washed leucocytes obtained from any source, and of a suspension of the bacteria to be tested are mixed and incubated at 37.5° C. Smears are then made from the mixture and after appropriate staining the average number of bacteria ingested per leucocyte is determined. This number represents the opsonic power of the serum and is sometimes spoken of as the phagocytic index. At the same time a similar specimen is prepared, only the serum used is that of a person known to be normal so far as that organism is concerned.

The ratio which the phagocytic index of a given serum bears to the phagocytic index of a normal serum is the opsonic index.

For example: Suppose the average number of bacteria taken up by the leucocytes in a preparation in which patient's serum has been used is 3, and the average number taken up by the leucocytes in a preparation in which normal serum has been used is 6, the ratio would be 3 : 6, and the opsonic index 0.5. Had the average number in the preparation in which patient's serum was used been 9 the opsonic index would be 1.5.

COMPLEMENT FIXATION

The principles which underlie the complement fixation tests are those first demonstrated by Bordet and Gengou, viz., that if antigen and its specific amboceptor are brought together in the presence of complement, the complement enters into combination with them; and if the antigen and amboceptor are present in sufficient amounts the complement is bound, or used up, in the combination. Evidence of this reaction is seen in all the cytolytic, bacteriolytic and proteolytic immune reactions. The part played by complement is probably that of an enzyme which acts upon the antigen causing its lysis provided a sensitizer specific to that antigen is present. Ehrlich's nomenclature of his third order of antibodies is used in the following discussions but this does not imply an acceptance of his conception of the mechanism of the reaction. This reaction of these 3 elements is so constant that any 2 of them may be used as a qualitative bio-chemical test to determine the presence or absence of the third. For example, given antigen (which may be any protein, cellular or in solution) and complement, and the presence of amboceptor specific to that given antigen may be detected. Given a specific amboceptor and complement and the presence of the antigen for which that particular amboceptor is specific may be detected. Given an antigen and its specific amboceptor and the presence of complement may be detected. In this way sensitive specific tests may be applied to the detection of meat adulteration, the character of blood stains, the identification of bacteria, etc., as well as to serological diagnosis. Thus, as originally shown by Bordet and Gengou, if an extract of typhoid bacilli and blood serum from a typhoid patient are brought together in proper proportions and incubated for a short time at body temperature, the complement in the serum, sensitized by the presence of typhoid amboceptor in the serum, will unite with the typhoid extract. If typhoid amboceptor is not present in this patient's serum, such union will not occur and the complement will remain free. Since it would not be possible to detect optically whether the complement remains free or becomes bound an indicator is necessary.

An indicator often used consists of washed sheep's erythrocytes and the serum of a rabbit which has been immunized against sheep corpuscles. If a suitable amount of this indicator is added after incubation to the tube containing the patient's serum and typhoid extract the presence of free complement would be indicated by hemolysis, *i.e.*, laking of the sheep corpuscles. Such a reaction would show that this particular patient's serum contains no specific amboceptor for typhoid bacilli to bind the complement to the antigen, *i. e.*, to the typhoid extract. If the patient's serum does contain amboceptor for typhoid bacilli, they together would bind or absorb the complement in their combination and then when the indicator is added no hemolysis, *i. e.*, laking, of the corpuscles would occur. The hemolysis is easily noted optically, and when the quantities of the reagents are properly adjusted this test may be made very delicate. Too great emphasis cannot be laid upon the quantitative adjustment of the reagents since the reliability and delicacy of all complement fixation depend very directly on this.

Glassware and Material.—The test tubes recommended for serological work measure 7 cm. long and 1 cm. in diameter. These hold approximately 6 c.c. Other sizes may be used if desired. Mohr pipettes of the following sizes are desirable: A 5 c.c. pipette graduated in 0.1 c.c., a 2 c.c. pipette graduated in 0.1 c.c.; a 1 c.c. pipette graduated in 0.01 c.c.; and one containing 0.2 c.c. graduated in 0.01 c.c. It is desirable that the graduations should in each case extend to the tip of the pipette. Graduated cylinders of 25, 50 and 100 c.c. capacity for making up solutions and glass beakers of 50 or 100 c.c. capacity are necessary.

The most convenient test tube rack is made of metal and holds a double row of 10 tubes each. The water bath should have a thermo-regulator capable of maintaining a constant temperature of 38°C. All glassware used should be reserved for this work only and should be kept scrupulously clean. After use the tubes and pipettes should immediately be emptied, rinsed in tap water, then either boiled or allowed to stand several hours in distilled water. Then the tubes may be inverted in a wire basket until dry. It is our practice to sterilize the glassware with dry heat as a final step in preparation, though bacteriological asepsis is not necessary. Any glassware which becomes permanently clouded should be discarded. Especial care must be used to wash every trace of reagents as well as of chemical cleaning solution from the pipettes before drying them.

Preparation of Hemolytic Amboceptor.—Because of their use as indicators in all complement fixation tests hemolysins have a great practical value. They are produced by injecting into one animal the red corpuscles of an animal of another species. Rabbits are the animals most commonly used for this purpose. They vary widely in their power to form hemolysins, but form a very potent hemolytic amboceptor when injected with sheep's corpuscles. Human blood, beef blood or that of fowls may be used. In many laboratories human blood is used because it is so easily obtained, yet it is more difficult to produce powerful hemolysins with human than with sheep corpuscles.

The sheep's blood may be obtained from the jugular vein of the animal by means of syringe, or it may be secured from an abattoir. It should be received into a clean sterile flask containing glass beads and shaken vigorously for 5 minutes to remove the fibrin, or it may be received into an equal quantity of isotonic salt solution containing 0.5% of sodium citrate to prevent clotting, then centrifugalized and the serum drawn off. The corpuscles are next freed of all serum by washing and centrifugalizing them 3 times, using each time from 5 to 10 times their volume of physiological salt solution.

Hemolysins appear in the rabbit's blood following the intra-peritoneal or intravenous injections of the washed corpuscles. Aseptic technic should be employed throughout. It is advisable to filter the corpuscle suspension in order to remove small clots of fibrin. The potency of the amboceptor produced bears no direct relation to the size of the doses of corpuscles injected; for example, a highly potent hemolytic serum may be prepared by three intravenous injections at intervals of 3 days of 3 c.c., 5 c.c., or 7 c.c. of a 10% suspension of sheep corpuscles. After from 6 to 10 days following the last injection a specimen of blood should be obtained from the rabbit's ear and the serum tested for its hemolytic power (see below).

Some, using a slower method, inject larger doses of the sheep's corpuscle and at longer intervals. For example, they inject 3 c.c., 5 c.c., 10 c.c., 15 c.c., and 20 c.c. respectively of a 10% corpuscle suspension at intervals of 6 or 7 days.

Instead of injecting the corpuscles intravenously the same quantities may be introduced intra-peritoneally at the same intervals. This method has the advantage that the animals are not so apt to die from anaphylaxis as when the intravenous method is used.

In the preparation of anti-human amboceptor Noguchi advises to give 4 injections of 4 c.c., 3 c.c., 4 c.c., 3 c.c., and possibly another of 4 c.c., at intervals of 4 or 5 days.

In immunizing rabbits against corpuscles it is advisable to use 2 or more animals simultaneously, as it frequently happens that only 1 of several will produce a highly potent serum.

One week after the last injection about 1 c.c. of blood is drawn from the ear of the rabbit and its serum titrated to determine its hemolytic strength. This may be done as follows:

In 1 row of clean test tubes is made a series of dilutions of the rabbit's serum with isotonic salt solution; *e. g.*, 1:25, 1:50, 1:75, 1:100, 1:200, 1:300, 1:400, 1:500, etc. Next, 0.1 c.c. from each of these dilutions is carefully measured into a corresponding row of empty tubes. This row then contains 1/250 c.c., 1/500 c.c., 1/750 c.c., 1/1000 c.c., etc., of the original rabbit serum. Two units of standardized (see page 573) complement are now added to each tube. If none is available 0.02 c.c. of guinea pig serum may be assumed to contain about 2 units of complement and this quantity used in testing the hemolytic serum. These small quantities are most easily obtained by diluting the serum 1:15 and then measuring 0.1 c.c. into each tube, or by diluting it 1:10 and adding 0.2 c.c. into each tube. Next 0.5 c.c. of a 2% suspension of washed sheep corpuscles (page 573) are added to each tube, and sufficient salt solution to make the total volume in each exactly 1.0 c.c. This is done that the reagents may act in uniform volumes. The set of tubes is now incubated for 30 minutes in a water bath at from 37°C. to 40°C. temperature, and the amount of hemolysis in each tube then noted. The smallest quantity of rabbit serum in this series which will cause complete hemolysis is accepted as 1 unit of amboceptor and twice that amount is the dose of this serum to be used in all hemolytic work. For example, if hemolysis is complete in all of the first 6 tubes in the above series and only partial in the others, then 1/3000 c.c. is the unit of amboceptor, since the 6th tube contained 0.1 c.c. of rabbit serum diluted 1:300, *i. e.*, 1/3000 c.c. of the original serum. It very frequently happens that the hemolytic amboceptor is so very potent that the above series of dilutions will have to be extended in order to determine the potency of the serum. Amboceptor whose unit is 1/50,000 c.c. or less is occasionally produced. It is our practice to discard amboceptor whose unit is a larger amount than .001 c.c. If the test shows the serum to be of satisfactory hemolytic potency, the rabbit is bled to death under aseptic precautions and the serum separated. It is our practice to introduce a No. 20 needle attached to a 30 c.c. syringe into the heart of the anesthetized animal since it is easier to secure the blood free from bacterial contamination by this method than by opening the carotid artery.

The rabbit serum may be preserved in small sterile tubes or ampullæ hermetically sealed, or dried on the filter paper. The most satisfactory method is to mix it with an equal quantity of glycerine to protect it against bacterial growth. This does not in any way affect the serological properties, for the serum will retain its potency for many months if kept in a cool place protected from light.

THE PREPARATION AND STANDARDIZATION OF COMPLEMENT.—Complement would seem to be a proteolytic enzyme which is normally present in the serum of the mammalia. Its strength or quantity differs in different individuals and in different species. It is destroyed easily by heat, by chemical substances such as acids, alcohol, etc., and by bacterial growth. It rapidly becomes weaker and finally disappears if the serum is allowed to stand exposed to warmth and light.

According to some methods of complement fixation use is made of the complement present in the patient's own serum. This is open to the objections that its amount varies so widely in different individuals and in the same person in disease conditions that to determine the strength of each patient's own complement at the time we planned to use it would involve much unnecessary work.

Complement is most active and least variable in amount in the serum of guinea pigs, hence this is the accepted source. Only vigorous healthy animals should be used, and especially animals which have not been used for any other purposes. It is advisable to use the pooled serum of 2 or more animals. The blood may be obtained either by opening the carotid artery and bleeding the animal to death or by introducing a No. 22 needle directly into its heart and withdrawing 4 or 5 c.c. of blood into a syringe. The latter method has the advantage of economy of animals since they may be bled repeatedly at intervals of 2 or 3 weeks. The blood is allowed to stand in a perfectly clean tube at room temperature until firmly clotted, the clot then loosened from the walls of the tube and the tube then placed in an ice box while the serum is allowed to separate. Or, the serum may be separated by centrifugation immediately after drawing the blood. The serum should be carefully removed from the clot or sediment so that it may be quite free from cells and hemoglobin.

In all work which involves the complementary property of animal serum it is very important that definite amounts of complement be used. Careful titration of the complement is therefore a most important preliminary step. The unit of complement is the smallest quantity which will produce complete hemolysis of the standard unit of blood cells mixed with two units of hemolytic amboceptor. It therefore depends directly upon the quantity of corpuscles used in the hemolytic system.

In the test as originally performed by Wassermann 1.0 c.c. of a 5% suspension of sheep corpuscles was the unit, or *N*, used. This was soon reduced to ½ that quantity (0.5 *N*), while now it is our practice to use 0.5 c.c. of a 2% suspension (0.2 *N*) of washed sheep corpuscles; that is, the equivalent of 1.0 c.c. of a 1% suspension. That is, the unit of complement we use is ⅕ the amount originally used and ⅖ of that recommended by many serologists. The advantage of a small unit of complement is that it greatly increases the delicacy of the test. Often the specific antibody for which we are testing is present in the patient's serum in very small amounts. If, therefore, there is scarcely enough antibody present to bind a quantity of complement represented by 0.2 *N* in the presence of the appropriate antigen, there would obviously be insufficient antibody to bind the amount represented by 0.5 *N* or *N*. Therefore a definitely positive reaction with the smaller unit of complement would be found negative if tested by a system in which a larger unit of complement was used.

The hemolytic system consists of complement, red blood-cells and amboceptor specific to those cells. Both the amboceptor and the complement are substances which vary in strength. The amount of blood corpuscles is the only easily controlled factor and hence is the unit to which the hemolytic system is adjusted. In order to provide a corpuscle suspension of as nearly constant value as possible, we add about 5 c.c. of sheep blood to about 45 c.c. of 0.85% salt solution, mix thoroughly and centrifugalize. This washing and centrifugation are repeated twice. In the centrifugation both speed and time are controlled, *i. e.*, 2000 revolutions per minute for 15 minutes. This will give a corpuscle sediment of almost constant density. One cubic centimeter of this sediment is carefully measured and made up to 50 c.c. with salt solution. This gives a fairly accurate 2% corpuscle suspension with which both complement and amboceptor may be standardized.

Titration of Complement.—A small amount of fresh guinea pig serum, obtained as described above, is diluted 1:20 with salt solution. Into a series of clean test tubes the following quantities of diluted complement are carefully measured, using a 1 c.c. pipette graduated in hundredths: 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.25 c.c., 0.3 c.c., 0.35 c.c., 0.4 c.c., etc. To each tube are then added 2 units of hemolytic amboceptor, determined as described on page 570, and 0.5 c.c. of 2% washed corpuscle suspension. A sufficient quantity of salt solution is now added to bring the total volume in the tube up to 1.0 c.c. The set is then incubated in a water bath for 30 minutes after which time the amount of hemolysis in each tube is noted. The smallest quantity of complement which produced complete hemolysis of the corpuscles is accepted as one unit of complement. Thus, in the above series, if hemolysis was only partial in the first 2 tubes and complete in the third and in all the remaining tubes, the unit of complement would be the amount present in the third tube, which is 0.2 c.c. of complement diluted 1:20, or 0.01 c.c. of undiluted complement.

Those who prefer the larger unit of corpuscles (see above) make the dilutions of complement as above, but add 0.5 c.c. of a 5% suspension of corpuscles and 2 units of amboceptor.

Whatever hemolytic system is used the density of the corpuscle suspension should be maintained as uniform as possible and that exact minimal amount of complement and amboceptor determined which will produce complete hemolysis of the unit of corpuscles used.

The Patient's Serum.—The serum is obtained by introducing a needle into a superficial vein and drawing the blood directly into a glass syringe. The vein usually chosen is the median cephalic on the anterior surface of the arm. The skin is sterilized by soap and water followed by an alcohol and iodine solution, or by Harrington's solution. The needle should have a caliber not larger than No. 21 and should be sterile. It is essential that the syringe be absolutely clean, dry and free from any trace of chemical substance. For this reason the syringe should not be washed with alcohol. A rubber tourniquet is applied above the elbow sufficiently tight to produce distention of the veins. The needle is inserted with the shank almost parallel to the skin surface, and with flat side of the point up. From 2 c.c. to 5 c.c. of blood is drawn into the syringe, the tourniquet then removed and the needle withdrawn. Pressure is made at the point of puncture with a sterile sponge or cotton immediately as the needle is withdrawn. The blood is transferred at once to a clean, dry tube and allowed to stand at room temperature until clotted. The clot is then separated from the sides of the tube and the specimen placed in a refrigerator for several hours. Usually a sufficient quantity of clear serum can be pipetted directly from the tube; if not, the specimen may be centrifugalized and the clear serum drawn off.

Before testing the serum it is necessary to destroy all of its complementary property. While many recommend that the serum be heated for 30 minutes at 56° C. we have found that heating it for even 5 minutes at 55° C. will completely inactivate the complement and hence have adopted the routine of heating the serum for 10 minutes at 55° to 56° C. In this way we avoid changing by the prolonged heating other properties of the serum than the complement.

It should be the rule to draw the patient's blood for serological tests before meals, or at least several hours after the last meal, since the products of recent digestion may render the serum anti-complementary. The presence of water, alcohol or other chemical in the glassware used may have a similar effect, or may produce sufficient hemolysis to make the serum unstable. The growth of bacteria in serum frequently produces anti-complementary properties and sometimes false positive reactions. For these reasons strict cleanliness should be observed in handling the serum and the tests should be made as soon as possible after the blood is drawn. It should be an invariable rule to keep all serological reagents in a refrigerator at a temperature slightly above 0° C. until they are used.

The Wassermann Test.—The antigen-amboceptor-complement [106] reaction was at first used for the detection of antibodies in sera and for the identification of bacteria. It was found to be exquisitely delicate, detecting minute amounts of antigens with the sharpest specificity limits of all the immunity reactions. It can even be used to determine the presence in tissues of specific organisms which cannot be cultivated; *e. g.*, the presence of a specific scarlatinal virus in the tissues of a patient with this disease. This fact led Wassermann, using as antigen extracts of the livers of congenital syphilitic fetuses, which contain great quantities of spirochetes, to attempt with this test to determine in a given serum the presence of specific amboceptors for the virus of syphilis, such amboceptors being present in persons infected with syphilis as a result of their reaction to the infection. As originally introduced, then, the Wassermann reaction was supposed to be a specific reaction, similar to the original complement fixation reaction of Bordet and Gengou between syphilitic antigen, specific syphilitic amboceptors and non-specific complement. It was soon discovered, however, that the reaction as it occurred in syphilis was decidedly different, since in place of a specific antigen (the extracts of tissues containing the syphilitic virus) it is possible to substitute the most varied sorts of tissue extracts which certainly are free from spirochetes (*e. g.*, ox heart). Now, syphilitic tissues are seldom used. One may even use with fairly satisfactory results commercial lecithin or mixtures of commercial lecithin and sodium oleate. Noguchi and Bronfenbrenner conclude as follows: "We know merely this: that complement in the presence of syphilitic antigen may be rendered inactive by one or more substances in the body fluids of a syphilitic or parasyphilitic patient."

Extended investigation of these non-specific "antigens" which give specific complement fixation with syphilitic sera has shown them to be related to the lipoids, especially the lecithins, as indicated by the fact that the most efficient "antigens" contain the acetone-insoluble fraction of the tissue lipoids. The antigenic value of this fraction of different liver extracts varies almost directly with its power to combine with iodine (Noguchi and Bronfenbrenner), which would indicate that unsaturated fatty acids are important in the reaction. Lecithins from different sources vary in efficiency; heart lecithin is more active than liver lecithin while brain and egg yolk lecithin follow. The addition of cholesterol to the lecithin solutions greatly increases their activity. An acetone-precipitated "antigen" of this class certainly is not a true antigen, for fixation antibodies are not developed in animals injected with those very lipoids which have been shown to be quite efficient in the Wassermann reaction.

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[106] Modified from Wells, Chem. Path., pp. 235–237.

The substance in the syphilitic serum which participates in the Wassermann reaction would seem to be related to the globulins, especially euglobulin, which are decidedly increased in the blood and spinal fluid of syphilitics.

P. Schmidt explains the reaction as due to the physico-chemical properties of the globulins of the syphilitic serum, which, he believes, possess a greater affinity for the colloids of the antigen than do normal globulins; this affinity is held in check in normal serum by the albumins of the serum, which in lues are relatively or absolutely decreased.

A favorite interpretation of the Wassermann reaction, which seems to harmonize with the known facts, is that there is a precipitation of serum globulin by the lipoidal colloids of the antigen and absorption of the complement by this precipitate.

Wassermann [108] and his collaborators also used salt-solution extracts chiefly of the spleen of a syphilitic fetus. The tissue was cut into small pieces. To 1 part by weight of this substance were added 4 parts of normal salt solution and 0.5% of carbolic acid. This was shaken in a shaking apparatus for 24 hours and then the coarser particles removed by centrifugation. The reddish supernatant fluid was used as the antigen. This could be preserved for a long time in dark bottles in the ice chest. Alcoholic extracts of syphilitic organs were later used by several authors who extracted syphilitic liver tissue for 24 hours with 5 times its volume of absolute alcohol. This was filtered through paper and the alcohol evaporated *in vacuo* at a temperature not above 40° C. About 1 gram of this material was then emulsified in 100 c.c. of salt solution to which 0.5% of carbolic acid had been added.

THE ANTIGEN.—While the term "antigen" as ordinarily used in the Wassermann reaction is, strictly speaking, a misnomer (see above) yet it is so generally used that it may be retained, but with a distinct understanding as to its actual meaning.

It is also of interest that in practical work this artificial antigen is even superior to the real antigen since the pure Treponema pallidum extract gives reactions in only a few late tertiary cases and results which run not at all parallel to the fixations obtained with non-specific lipoidal substances. Although we are, at the present writing, still in the dark as to whether the syphilitic antigen depends for its properties upon the lipoidal nature of the extracts, or upon the size and dispersion of the particles present in the extracts, we can still assert that the test is reliable and, with care in execution and interpretation, is of enormous value in the diagnosis of syphilis. It is necessary, however, to recognize that it surely is not a specific antigen-antibody reaction.

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[108] Modified from Hiss and Zinsser, pp. 263–264.

The antigens in common use to-day are prepared as follows:

1. Beef, sheep, human or guinea-pig heart muscle finely chopped up and extracted in 5 times its volume of absolute alcohol. This mixture is kept for from 5 to 7 days in the incubator, during which time it is frequently shaken. It is then filtered and titrated. This preparation of antigen is known as " plain alcoholic extract of heart muscle."

2. *Noguchi's Acetone-Insoluble Lipoid Antigen.*—Fresh spleen or heart muscle is macerated and extracted for from 5 to 7 days in the incubator in 5 times its volume of absolute alcohol, during which time it is frequently shaken. It is then filtered and evaporated to dryness with the aid of a fan. The sticky residue is taken up in a small quantity of ether and this ether solution poured into 4 times its volume of acetone (C. P.). The floccular precipitate which forms is collected and can be preserved under acetone. About 0.2 gms. of this paste is dissolved in 1 c.c. of ether and, added to 9 c.c. of pure methyl alcohol for permanent preservation. It is diluted with salt solution before using

3. *Cholesterinized Antigen.*—According to the researches of Sachs and Rondoni, Browning and Cruikshank, and Walker and Swift antigen can be made more delicate by the addition of cholesterin. Walker and Swift recommend that to an alcoholic extract of human or guinea-pig heart enough cholesterin be added to make a concentration of 0.4%.

Whichever antigen is used it must be tested and known to conform to the following requirements:

It must show no anticomplementary nor hemolytic action when used in small amounts.

It must be sensitive to the presence of syphilitic antibodies, as shown by the absorption of complement when mixed with the serum from known syphilitic patients.

It must not absorb complement when mixed with non-syphilitic serum.

There must be a wide margin between the antigenic dose and the amount ot the antigen which by itself would manifest anticomplementary or hemolytic action.

It must keep well and develop no variations in the above characteristics.

Different extracts prepared after the same manner should be quite similar in their characteristics.

The suitability of the antigen is determined as follows:

To estimate the degree of its anticomplementary action the following quantities of the antigen are carefully measured into a series of clean, dry tubes: 0.25 c.c., 0.1 c.c., 0.075 c.c., 0.05 c.c., 0.025 c.c., 0.01 c.c., 0.0075 c.c., 0.005 c.c. Two units of complement determined as described on page 573 are added to each of these tubes and also to a control tube containing no antigen. The total quantity in each tube is made up to 0.5 c.c.

with normal salt solution. These tubes are incubated for 30 minutes at 38° C., then 2 units of amboceptor and 1 unit of washed corpuscles appropriate to the amboceptor are added to each tube and the tubes are again incubated for 30 minutes. If in the control tube there is complete hemolysis and there is failure of hemolysis in the remaining tubes, then this antigen certainly has the property of inhibiting the action of complement. If there is no hemolysis in the first 2 tubes, partial in the third and complete hemolysis in the remaining tubes, then this antigen is anticomplementary in quantities of 0.075 c.c. or more.

To determine whether the antigen itself is capable of causing hemolysis, a series of tubes is arranged which contain the same quantities of antigen as above. No complement is added to any of the tubes in this case. The volume of each tube is now made up to 0.5 c.c. with salt solution and a unit of corpuscle suspension added to each tube. The tubes are now incubated for 30 minutes and the results read as before. Any hemolysis noted in this series indicates that the antigen possesses hemolytic property and if it is present in the tubes containing the small quantities of antigen, this antigen must be discarded as unsatisfactory.

To determine the sensitivity or antigenic property of the antigen the following quantities of antigen are carefully measured into a series of clean dry tubes: 0.05 c.c., 0.025 c.c., 0.01 c.c., 0.0075 c.c., 0.005 c.c., 0.0025 c.c. Sufficient salt solution is added to each to make the total amount 0.3 c.c. To each of these tubes is next added 0.1 c.c. of inactivated patient's serum which has previously been tested and is known to give a positive test, also 2 units of complement determined as previously described. The following controls also are prepared: One tube containing the patient's serum, 2 units of complement and sufficient salt solution to make the total volume 0.5 c.c. and 1 tube containing 2 units of complement alone and made up to 0.5 c.c. with salt solution. These tubes are incubated in a water bath for an hour at 38° C. Then 2 units of amboceptor and 1 unit of corpuscle suspension are added to each tube and they are incubated again for 30 minutes. Should there be no hemolysis in the first 4 tubes and partial or complete in the others it would indicate that the antigen is sensitive, since 0.01 c.c. will bind complement in the presence of syphilitic serum. If, as in this illustration, the amount of the antigen which will bind complement is less than ¼ the quantity which is either anticomplementary or hemolytic then the antigen is satisfactory. It usually is the case that satisfactory antigen is sensitive in smaller quantities than illustrated in this instance. Antigen should never be used whose antigenic dose approaches the anticomplementary or the hemolytic quantity by a narrower margin than that illustrated in the above example.

It is well to run each new antigen which has been prepared and tested as above described in parallel tests with an antigen previously used and known to be sensitive on a rather large series of routine cases before adopting the new antigen for routine work.

The quantity of antigen used in routine work should not exceed twice the minimum sensitive quantity determined as above since it has been found that occasionally antigen in excess is not as sensitive as is the minimum quantity.

It is the custom in many laboratories always to use 2 or more antigens in routine work. If only 1 antigen is used, we prefer the acetone-insoluble fraction of the alcoholic extract of beef heart as prepared by Noguchi, since we have found that this usually is more delicate than plain alcoholic organ extracts.

The cholesterinized antigen, recommended by many workers because it is so sensitive, may be too sensitive since the blood of individuals who seem clinically quite free of syphilis has given positive tests with this antigen and negative to all other used. It is the opinion of many workers that the cholesterinized antigen is most useful in tests which are intended to control the results of the treatment of cases known to have syphilis. If it is used as an antigen in routine diagnostic work, other tests using another antigen should be run in parallel with it. For illustration, if a case gives a weakly positive reaction with alcoholic extract or acetone-insoluble antigen, and a strongly positive one with the cholesterinized antigen then the suspicion of syphilis is strengthened.

Technic of the Wassermann Test.—Since the technic originally used by Wassermann is the basis of all modifications now in general use his method of performing it will be briefly outlined. Wassermann used as antigen an aqueous extract of the liver of cases of congenital syphilis. This antigen is rarely used now and any one of the antigens described above may be substituted for it. His reagents were prepared as above described except that the dose of hemolytic amboceptor was determined for a larger quantity of sheep cells. As many pairs of tubes were placed in a rack with a double row of holes as there were patients' sera to be tested, each pair consisting of 1 tube in the front row and the one just behind it. Three additional pairs were added for controls. Into all the tubes was measured 0.7 c.c. of salt solution and then into each pair 0.2 c.c. of an inactivated patient's serum. This was done with each of the sera to be tested and also with a serum known to be positive and one known to be negative. Into each tube, front and back, was placed 0.1 c.c. of fresh guinea pig serum; then 1 c.c. of antigen so diluted that this quantity contained twice its minimal antigenic dose (see page 578). The additional tube in the front row contained antigen and complement alone and served as an antigen control. The corresponding tube in the rear row contained only complement and served as a control on the hemolytic system. The volume in each tube was now brought to a total of 3 c.c. by the addition of salt solution and the tubes all incubated at body temperature for 1 hour. Following the incubation 1 c.c. of amboceptor, so diluted that this quantity contained 2 hemolytic units, and 1 c.c. of a 5% suspension of washed sheep corpuscles were added to each tube. After another incubation period of 1 hour the results were read. It was the practice in some laboratories to place the racks in an ice box for 2 or 3 hours after the second incubation period. This will facilitate the estimation of the hemolysis, particularly in tubes where it may be only partial, since on standing the non-hemolysed corpuscles will settle to the bottom of the tubes.

A widely used modification of the above technic consists in using the reagents in exactly ½ the quantities specified above.

The method we now are to describe may be regarded as the original Wassermann technic except that ⅕ the quantities of that technic are used. The essential variations are the extreme care used in adjusting the hemolytic system, the fact that ½ the original quantity of the patient's serum is used and ⅕ the quantity of the other reagents. This increases the proportion of patient's serum to the other reagents and so tends to increase the delicacy of the test. We believe that this is the most satisfactory for routine use of all of the modifications of the Wassermann test. When carefully performed it combines a high degree of delicacy with a relatively simple technic. It is adapted to all complement fixation tests, as well as to that for syphilis. The directions given above for titrating the various reagents are intended for this modification alone. If other modifications are used the same scheme of titration may be employed, only one makes suitable changes in total volumes to adapt the quantities to those of the modification in question.

The antigen, complement, amboceptor and washed sheep corpuscles are prepared and titrated as described on the preceding pages. The patient's serum, free from red blood corpuscles, is inactivated at 55° for 10 minutes. As many pairs of tubes are placed in the rack as there are sera to be tested. If several, time is saved in pipetting and greater accuracy in measurement is obtained if the antigen, complement and salt solution are mixed together in suitable proportions and the proper quantity of this measured into each tube. For illustration, if 16 sera are to be tested, an antigen-complement mixture for 20 (allowing for controls) is made as follows: Assuming for example that the unit of complement (see page 574) is .0075 c.c., since 2 units are used in each test then 40 such units, that is 0.3 c.c., is measured carefully into a dry, clean, small graduate. Assuming further that the unit of antigen (see page 578) is 0.01 c.c., since 2 units are used in each test then 40 units, or 0.4 c.c., is measured accurately into 5 or 6 c.c. of salt solution and added to the 0.3 c.c. of complement. The total volume of complement antigen mixture is now brought to exactly 8 c.c. with salt solution and the whole well mixed. Then 0.4 c.c. of this complement-antigen mixture is measured carefully into each of the twenty tubes of the front row in the racks. Diluted complement without antigen is prepared in the same proportion as above and 0.4 c.c. pipetted into each tube in the rear row. A 2 c.c. or a 5 c.c. pipette graduated in tenths is convenient for this measurement.

The "set-up" is now ready to receive the sera to be tested. One tenth of a cubic centimeter of each serum is added to 1 tube in the front row and to the corresponding tube in the rear row. Then the pipette is rinsed with salt solution and the same amount of another serum added to each of the next pair. To the seventeenth pair of tubes is added the same amount of a serum which a previous test has shown to be definitely positive and to the eighteenth, a serum known to be negative. This leaves 2 pairs of tubes to which no human serum is added for controls of the reagents, the front tubes for complement-antigen and the rear tubes for complement. To each of these controls one adds 0.1 c.c. of salt solution (in place of a serum) in order to make the volume in all tubes equal.

The "set-up" is now incubated in a water bath for an hour at 38° C., after which time sheep cells and their specific amboceptor are added as a test for the presence of free complement. Two units of amboceptor (see page 571) and 0.5 c.c. of 2% corpuscle suspension are added to each of all the tubes. Time may be saved if enough corpuscles and amboceptor for all are first mixed. That is, 0.5 c.c. of corpuscles from the centrifuge tube (see page 573) are measured with a wet pipette into 24.5 c.c. of salt solution. This amount is sufficient for 50 tubes. Since each tube must receive 2 units of amboceptor 100 units of this are added to the corpuscle suspension. For example, if the titration of amboceptor as described on page 571 shows that .0015 c.c. is the unit, then 100 times this amount, or 0.15 c.c. of undiluted amboceptor is added to the sheep corpuscles and thoroughly mixed. Then 0.5 c.c. of this mixture is measured into each tube and they all are again incubated. Most authors specify 1 hour for this last incubation time, but if the reagents are satisfactory hemolysis is complete before 30 minutes and further incubation produces little change.

In performing Wassermann tests on cerebrospinal fluid it is not necessary to inactivate this fluid since it contains no complement. A larger quantity of spinal fluid is used than in the case of blood serum. It is our custom to use 3 pairs of tubes for each spinal fluid to be tested. The tubes in the front row contain antigen, complement and salt solution, and those in the rear row complement and salt solution only, just as in the tests on serum. To each pair of tubes are added respectively 0.2 c.c., 0.5 c.c. and 1.0 c.c. of the spinal fluid. The rear row of tubes serves as a control on any anti-complementary property which may be present in the fluid. In these control tubes, of course, the hemolysis should be complete. The results are noted and recorded exactly as described for patient's serum.

When reading the results one first studies the controls. The corpuscles in the antigen-complement controls and in the complement controls should be completely hemolysed. The corpuscles in the tubes containing known negative serum should be completely hemolysed. The front tube of the pair containing a known positive serum should show no trace of hemolysis and its rear tube should show complete hemolysis. Any deviation from these results in the controls indicates that the results of none of the tests are dependable. In the case of the unknown sera a failure of hemolysis in the rear tube indicates that that patient's serum has properties which inhibit the action of complement, or is "anti-complementary." No result either positive or negative therefore can be recorded. If the corpuscles in the rear tube of a pair are completely hemolysed and there is no hemolysis or only partial hemolysis in the front tube, the result is read "positive." It is a common custom to read a result as ++++ if the corpuscles in the front tube show no trace of hemolysis; to read +++ if there is very slight hemolysis; ++ if approximately half hemolysis has occurred; and to read + if hemolysis is almost complete.

The above is the procedure for testing a series of 16 sera with one antigen using the method for adjusting the reagents which we have found more delicate than many methods now in practice and which at the same time is not impractical since too time-consuming. If it is desired to use more than one antigen a third parallel series of tubes containing the second antigen, prepared exactly as described for the front row in the above series, is added to the "set-up."

Interpretation.—It has been said that the strongest evidence of the value of the Wassermann test is the fact that now, but especially during the earlier period of its use, the results obtained by workers, many of whom possessed little laboratory skill and less scientific training, were sufficiently consistent with the clinical diagnosis to establish the test in the position which it holds; that is, the most valuable single laboratory test of the presence of syphilis. The question of the interpretation of the results obtained with the test has caused much debate among clinicians. Some hold, not without justification, that since the same serum tested separately in 2 or more laboratories may be reported with contradictory results, they cannot be expected to place reliance upon the test. This emphasizes the fact that before the question of its value can be discussed the qualifications of the serologist must first be considered. There are so many factors entering into the test and so many possibilities for error that unless the worker is thoroughly trained in the principles of serology and is conscientious and painstaking in his technic he may easily bring the test into disrepute. In discussing the interpretation of results, therefore, it is assumed that they were obtained by a method which is reliable and by a worker of unquestionable accuracy. It is interesting that where such conditions obtain the number of reports of weakly positive reactions is reduced to a minimum and the most are definitely negative or strongly positive. Of course some sera will give a partially positive test even with most careful technic. The interpretation of these will be discussed later.

Noguchi collected the results of a number of investigators who used the Wassermann reaction in the diagnosis of syphilis. The figures obtained by him are briefly summarized here since they still hold.

Condition	Number of cases	Percentage positive
Primary syphilis	416	69.8
Secondary syphilis manifest	1605	89.4
Tertiary syphilis manifest	581	78.1
Early latent syphilis	1233	51.
Late latent syphilis	861	47.
Hereditary syphilis	125	94.5
Cerebrospinal syphilis	64	47.6
General paralysis	498	88.1
Tabes	216	62.66

Noguchi reported a higher percentage of positive reactions in syphilis and the parasyphilides with his method of performing the test than with Wassermann's method.

While the Wassermann reactions may be positive in a few conditions other than syphilis, this is not nearly as common as the earlier reports would indicate. The test is sometimes positive in frambesia (a disease caused by an organism very similar to that of syphilis), in malaria, in some cases of relapsing fever and in leprosy of the tuberculous type. Chloroform or ether anesthesia is occasionally followed by a positive Wassermann test in otherwise normal individuals. The early statement that the blood in scarlet fever sometimes gives a positive test has not been substantiated, for now it is found uniformly negative except in cases where syphilis cannot be ruled out; and the same is true of pellagra.

Malaria with Positive Wassermann.—J. S., No. 6858, aged 13 years, was admitted with a diagnosis of tuberculous coxitis (left). The high fever, sometimes accompanied by a chill, suggested malaria and blood examination demonstrated Plasmodium vivax. On Sept. 21, 1918, the Wassermann test of the blood was 3 plus and Sept. 28th it was 4 plus. Quinine was started September 22nd. On both Oct. 5th and 12th the blood Wassermann was negative.

Treatment with mercury or salvarsan may quickly cause the serum of a syphilitic patient to lose the power of giving a positive Wassermann reaction, so that for diagnostic purposes it is necessary to take this fact into consideration.

In cases suspected of cerebrospinal lues including tabes and paresis it is advisable to test both the serum and spinal fluid, since either one and not the other may prove positive.

With the exceptions of the conditions noted above the present opinion is that a positive Wassermann test indicates the presence of living spirochetes somewhere in the tissues of the patient.

Unfortunately a negative reaction cannot be interpreted as definitely as can one which is positive. Even with the most painstaking technic and using every modification of the test the reaction in a small percentage of known cases of syphilis in all stages is negative. It has been demonstrated repeatedly that an alcoholic debauch will occasionally produce a negative reaction in an individual whose serum previously gave a positive reaction. The percentage of negative tests varies much in different stages of the disease as well as in the reports of different workers. The percentage of these erroneous negative reports has become progressively smaller as the technic cf the test has improved but there is at present no reason to hope it will be reduced to zero.

It has been shown that the amount of the substance in the syphilitic patient's serum which absorbs complement in the presence of appropriate antigen varies widely. For illustration, in some cases of unquestionable luetic infection no such substance can be demonstrated, while in other similar cases it is present in amounts sufficient to absorb several times the quantity of complement ordinarily used in the test. It is easy to see that between these two extremes may be found all possible quantitative variations in the complement-absorbing power of the serum of a syphilitic patient. It therefore would not be strange if in certain cases of syphilis the serum contained enough of this substance to bind all the complement used in the test and in addition left enough free to cause partial hemolysis when the indicator is added. If the presence of this substance in quantity sufficient to absorb all the complement, and therefore give a + + + + positive reaction, is evidence of syphilis, then the presence of this substance in any detectable quantity whatever is similar evidence. Therefore, if the technic is above question, a + + or a + + + reaction should be regarded as of practically the same significance as a + + + + positive. A faint positive, as +, should, however, be interpreted as suggestive only.

Complement Fixation in the Diagnosis of Gonococcus Infection.—Very early in the development of the complement fixation test attempts were made to apply it to the diagnosis of gonococcal infection but these proved only partially successful. Later it was found that the gonococcus is not a single distinct strain or species, but a large group of closely allied strains of organisms which can be differentiated by the agglutination tests. The antigens used in the earlier experiments presumably were prepared from only one of these strains. Later investigators found that by using antigen containing each of the known strains of gonococcus—*i. e.*, polyvalent antigens—the complement fixation test gives satisfactory results.

The difficulty of isolating the gonococcus in pure culture and the care required in sub-culturing a large number of strains make the preparation of gonococcus antigen so tedious that most workers use an antigen prepared and standardized by a laboratory whose specialty is the preparation of biological products.

As a preliminary step the amount of the antigen necessary to show anticomplementary properties is carefully determined, using the same scheme as described for testing Wassermann antigens, and a quantity not larger than ¼ this amount is used in the test. Complement, amboceptor and sheep corpuscles are prepared and standardized exactly as for the Wassermann test. It is important that whatever hemolytic system is used it should be so adjusted that the amount of complement should be small in proportion to the amount of patient's serum used. For this reason, as in the Wassermann test, one-fifth the original Wassermann quantities are to be recommended. The patient's serum is inactivated as for the Wassermann test.

One pair of tubes is allowed for each patient's serum and 1 pair for each control. One-tenth of a cubic centimeter of complement so diluted that this amount contains 2 units, is measured into every tube in the series. One-tenth of a cubic centimeter of antigen, so diluted that this amount contains not more than ¼ the anti-complementary dose, is added to each tube in the front row. One-tenth of a cubic centimeter of each patient's serum is placed in each of the pair of tubes assigned to that test. To 1 pair of tubes is added a previously tested positive serum and into another pair a negative serum. An additional pair contains no serum, but the front tube antigen and complement in the above specified quantities, and the rear tube the complement alone. The total volume in each tube is brought to 0.5 c.c. by the addition of salt solution. The set-up is incubated in a water bath at 38° C. for 1 hour, after which 0.5 c.c. of a 2% suspension of sheep corpuscles and 2 units of the proper amboceptor are added to each tube. The incubation is repeated and the results recorded exactly as described for the Wassermann test.

The complement fixation test for gonococcus is a definitely specific test which depends on the presence of specific antibodies in the patient's serum. It therefore differs from the test for syphilis. The test is frequently negative in acute gonococcal urethritis, probably because the infection, being acute and local, has stimulated the production of less free antibody in the circulating blood than would one which has extended to other structures and which has been of longer duration. The highest percentage of positive results is obtained in series of cases of acute exacerbations of chronic urethritis, in involvement of the prostate or epididymis, in gonorrheal iritis, in salpingitis and in arthritis. In these conditions the serum of approximately 80% of the cases gives a positive test. A failure of the patient's serum to fix complement in the presence of gonococcus antigen, therefore, does not exclude gonococcus infection. A positive reaction may persist for several weeks after all evidence of the infection has disappeared.

Williams[109] found the test of particular value in strengthening one's suspicion of a gonococcal infection in cases in which the gonococcus could not be discovered bacteriologically, nevertheless the final proof of a gonorrheal infection is the cultivation of the gonococcus. As a matter of technic the cultivation of the gonococcus, owing to its susceptibility to cooling, its association with other more rapidly growing organisms and the need of special media, is much more difficult than the fixation test. Except in acute cases, where the gonococci are abundant, the examination of a Gram-stained smear should not be accepted as conclusive since Micrococcus catarrhalis, Diplococcus crassus, irregular types of Gram-negative cocci, and, most important of all, the so-called "degeneration" forms of staphylococci may lead to error.

In interpreting a positive test, it should always be borne in mind that gonorrhea is a very widespread disease and that an individual may suffer from at least two different infections.

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[109] Interstate Med. Jour.. xxi, 1914.

A positive reaction in a patient supposedly cured of gonorrhea indicates the presence of a gonococcal focus and his capability of infecting others. The importance of this in connection with problems of marriage is great. A positive reaction occurs in about 20% of those clinically cured.

In acute cases, in which the gonococci are usually easily demonstrated, the fixation test is generally negative. On the contrary in the chronic and ill-defined affections, where it is not often possible to obtain the organism, the test acquires its greatest sensitiveness, expecially in the diagnosis of gonorrheal arthritis and of gonorrheal epididymitis, at least by the fifth week, about 100% of which cases give positive reactions. About 75% of the cases of posterior urethritis, prostatitis and seminal vesiculitis with recurrent exacerbations, about 65% of cases of pyosalpingitis and about 65% of all stricture cases give positive reactions.

A negative reaction would not exclude gonococcal infection, especially in the acute and subacute stages without complications and when limited to the urethra or vagina.

Syphilis or a positive Wassermann reaction does not interfere with the test.

In gynecology the test has proved its value in the differential diagnosis of pelvic inflammatory diseases from one another and from neoplasms. The test is usually negative in uncomplicated cases of urethritis, vulvovaginitis and Bartholinitis; it appears that the infection must reach at least to the level of the uterus before a positive reaction develops.

Complement Fixation for Tuberculosis.—Attempts had been made to demonstrate complement fixation in cases of tuberculosis before Wassermann applied the test to syphilis. In these earlier attempts Koch's tuberculin and other similar preparations were used as antigen. Subsequently a great variety of preparations of tubercle bacilli and their products were used by various workers and with various results. The literature on this subject is too extensive to be reviewed here, but in brief it may be said that antigens prepared by widely different methods have given a fairly high percentage of positive tests in cases of tuberculosis, especially the early cases, and that the percentage of positives in normal individuals is small. Our own experience with the test has led us to believe that if extreme care is used, especially in adjusting the hemolytic system, the test is of real value in diagnosis. It should be regarded in the same manner as other laboratory tests, not as evidence on which alone the diagnosis of the patient's present illness may be established, but as evidence which, properly interpreted, will help. We have used antigens prepared after the methods of Miller, Bronfenbrenner and Petroff in a large series of cases and have found the results to conform closely with the results of the clinical examination.

The wide variation in the results obtained by various workers with this test is probably due to the fact that in tuberculosis free antibodies are frequently present in the blood in amounts too small to bind the relatively large amounts of complement which some use in complement fixation technic. For example, many use in the hemolytic system 0.5 c.c. of a 5% suspension of sheep corpuscles. Two of the units of complement which would correspond to this quantity of sheep cells would be a larger amount than could be absorbed by the amount of tuberculous antibodies usually present unless these happen to be present in unusually large amounts. If, on the other hand, the hemolytic system is based upon 0.5 c.c. of a 2% corpuscle suspension, two units of complement standardized for this quantity might be absorbed by the patient's serum in the presence of suitable antigen and result in a positive test. The above explanation has been justified repeatedly both in tests for syphilis and for tuberculosis by finding sera which react negatively or faintly positively with the larger units of complement but which completely bind the complement when a smaller unit is used.

The simplest method of preparing an antigen for the test for tuberculosis is that of Miller. A number of strains of tubercle bacilli are grown in glycerine broth, and the growths from the cultures are collected and mixed. Of this, 10 mgms. is mixed with 90 mgms. of dry sodium chloride and ground for an hour in an agate mortar. This amount is made up to isotonicity with distilled water and vigorously shaken. The supernatant fluid after the larger particles have settled to the bottom of the tube is the antigen. It is well for the sake of safety to sterilize this by heat before using it. The test for the anti-complementary property of the antigen, the preparation of reagents and the technic of the test are exactly the same as described for the Wassermann test.

It has been found that a considerable percentage of the serums which give a strongly positive Wassermann test will bind complement also with tuberculous antigens. This is spoken of as "cross fixation" and makes it necessary when applying the test for tuberculosis to test each serum first against a syphilitic antigen, otherwise a cross fixation might be mistaken for a positive tuberculous fixation. Stivelman [110] from the study of 700 cases considered the test of little clinical value since but 33% of the cases of definite incipient tuberculosis gave a positive test and since it did not assist in determining clinical activity or immediate prognosis. Petroff [111] believes this test even more specific than the Wassermann.

It should be pointed out that there is no necessary conflict in these 2 opinions. The test may be very specific and yet of little value. We take it for granted that a much larger percentage of persons have at some time of their lives had tuberculosis of which they were not aware and of which they now are well than is the percentage of those who have known of their infection because of outspoken symptoms. The question is, Have they now a tuberculous infection which they should fight?

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[110] The Jour. of Lab. and Clin. Med., April, 1920, v, p. 453.
[111] Am. Rev. of Tuberc., 1920, iii, p. 683.

ISOHEMAGGLUTININS

The presence of isohemagglutinins in human blood was discovered independently by Landsteiner and Shattuck in 1900. Since then extensive studies of these have been made by a number of workers. Landsteiner found that individuals may be grouped into 3 classes according to the behavior of their serums and corpuscles toward those of other individuals. A fourth group or class has been added by subsequent investigations. In 1910 Moss published an extensive study of isohemagglutinins. He divided individuals into 4 groups according to the behavior of their bloods and determined the percentages of individuals in the different groups. Unfortunately Moss and Landsteiner, whose results are practically identical, named their groups differently, Moss assigning to group four the individuals placed by Landsteiner under group one. Confusion inevitably arose from this situation.

Landsteiner's classification is as follows:

Group I.—The corpuscles of Group I are not agglutinated by any human serum; the serums of Group I agglutinate the corpuscles of each of the other groups.

Group II.—The corpuscles of Group II are agglutinated by the serums of Groups I and III; the serums of Group II agglutinate the corpuscles of Groups III and IV.

Group III.—The corpuscles of Group III are agglutinated by the serums of I and II; the serums of Group III agglutinate the corpuscles of Groups II and IV.

Group IV.—The corpuscles of Group IV are agglutinated by the serums of each of the other groups; the serums of Group IV do not agglutinate the corpuscles of any other groups.

According to the classification of Moss the groups are arranged as follows:

Group I.—The corpuscles of Group I are agglutinated by the serums of Groups II, III and IV; the serum of Group I does not agglutinate any corpuscles.

Group II.—The corpuscles of Group II are agglutinated by the serums of Groups III and IV; the serum agglutinates the corpuscles of Groups I and III.

Group III.—The corpuscles of Group III are agglutinated by the serums of Groups II and IV; the serum of Group III agglutinates the corpuscles of Groups I and II.

Group IV.—The corpuscles of Group IV are not agglutinated by any serums. The serum of Group IV agglutinates the corpuscles of Groups I, II and III.

These relationships may be graphically demonstrated by the accompanying charts:

LANDSTEINER

	Series I	Series II	Series III	Series IV
Corpuscle I	o	o	o	o
Corpuscle II	+	o	+	o
Corpuscle III	+	+	o	o
Corpuscle IV	+	+	+	o

Moss

Corpuscle I	o	+	+	+
Corpuscle II	o	o	+	+
Corpuscle III	o	+	o	+
Corpuscle IV	o	o	o	o

+ =Agglutination.
o =No agglutination.

The following approximate percentages are given to the different groups by Moss: Group I, 10%; Group II, 40%; Group III, 7%; and Group IV, 43%. It is stated that the type of blood of the individual is an inherited trait which is transmitted according to the Mendelian law and is permanent during the life of the individual.

The use of blood transfusion as a therapeutic measure has brought the subject of hemagglutinins into prominence since it is found that frequently serious results follow when donor and recipient are members of different groups.

It was formerly the custom to test the bloods of donor and of recipient for compatibility by mixing the donor's corpuscles with the recipient's serum and the donor's serum with the recipient's corpuscles and examining these mixtures for the presence of agglutination. If no agglutination appeared the bloods were pronounced compatible.

The above procedure, while perfectly satisfactory, has been superseded in large measure by the test for the determination of the group to which an individual belongs. To do this it is necessary to have serums known to belong to Groups II and III. Approximately 2 drops of the blood of the individual to be tested are suspended in about 1 c.c. of salt solution which contains preferably about 0.5% of sodium citrate. This gives a corpuscle suspension of suitable density. Upon a clean cover glass are placed a loopful of serum Type II and a loopful of serum Type III. A loopful of the corpuscle suspension is added to each and a third loopful of the corpuscle suspension is placed on the same cover slip as a control. A hollow ground slide rimmed with vaseline is inverted over the drops on the cover slip and after 5 to 10 minutes the preparation is examined under low power of the microscope. The presence of agglutination is easily detected by inspecting the corpuscles. It will be seen by reference to the above charts that the group of any individual's blood may be determined by the behavior of its corpuscles to the serums of Groups II and III. Thus, in Moss's classification, if the corpuscles are agglutinated by both serums II and III, the blood belongs to Group I; if the corpuscles are agglutinated by serum III and not by serum II, the blood belongs to Group II; if the corpuscles are agglutinated by serum II, and not III, it belongs to Group III; and if the corpuscles are agglutinated by neither serum it belongs to Group IV.

It is seen that the determination of the group to which donor and recipient belong is a simpler procedure than that of testing them for hemagglutination by crossing the serum of each with the corpuscles of the other. It has the additional advantage also that the type of blood of the recipient need be tested but once. Each prospective donor is then tested separately until one is found belonging to the same group as the recipient. The sera used for the agglutination test will keep for many weeks without deterioration.

THE BLOOD IN DISEASE

Anemia.—The popular definition of an anemia has been, a deterioration of the blood qualitatively and quantitatively as regards one or all of its constituents—the plasma, the corpuscles and the hemoglobin (Grawitz). While the red blood-cells are in fact a relatively unimportant part of the total blood compared with the plasma upon which depends the health of the whole body including the red corpuscles, yet their number and their hemoglobin are the only easy criteria for estimating the blood's condition and so practically the term anemia is limited to conditions of the blood which affect these cells, their number per cubic millimeter, their hemoglobin content, or both. Unfortunately these values relate to but 1 c.mm. of blood and not to the volume of blood as a whole.

The concept of anemia should include a diminution in the total volume of the blood but the estimate of this is as yet not practicable clinically although examinations at the autopsy table even in cases with a practically normal blood-count show that this volume does change considerably.

Many have tried to define anemia in terms of changes in the plasma but this has proved unsatisfactory. Some definite plasma changes may mean little while the plasma in the severest anemias (clinically) with the lowest counts may chemically be almost normal.

By *oligocythemia* or *hypocythemia* is meant diminution in the number of red blood-cells in 1 c.mm. of blood. This may be due either to an actual reduction in the total number of the red cells in the body, or merely to an increased volume of plasma. By *oligochromemia* is meant a diminution in the amount of hemoglobin as judged by its color compared with that of an equal volume of normal blood. By *color-index* is meant the percentage of hemoglobin divided by the percentage of the red blood-cells, 5,000,000 cells considered as 100% (see page 482).

By *oligemia* is meant a diminished total amount of blood in the body. This may be suspected, but as yet cannot be proved. *Oligemia serosa* is an oligemia of diluted blood; *oligemia sicca*, an oligemia with blood qualitatively normal. *Hydremia* means an increased percentage of water in the plasma and occurs whenever the albumin is diminished. *Polyplasmia* is an increase in the volume of the plasma, supposed to occur in chlorosis; *oligoplasma*, a decrease, which occurs in certain cardiac diseases. By *plethora vera* is meant an increase in the total volume of blood. This can only be suspected.

By the *hematopoietic organs* one usually means the organs furnishing the corpuscles; *i. e.*, the bone-marrow, spleen and lymph-glands. The bone-marrow certainly furnishes red corpuscles and many leucocytes; the spleen is active perhaps after a severe hemorrhage, but otherwise is probably unimportant in the production of red blood-cells. It is possible that some leucocytes originate there and quite probable that this organ removes some of the old cells. The function of the lymph-glands as hematopoietic organs is still in doubt.

The red blood-cells have the indispensable yet simple function of transporting the oxygen to the tissues. The leucocytes are thought to be important in immunity production and in the absorption of neutral fat in the intestine, while by their disintegration they certainly raise the albumin content of the blood. The function of the platelets, apart from their possible relation to coagulation, is not understood. While these functions of the formed elements are very important those of the plasma of the blood are far more intricate and varied. We study the former because they are as yet the only practical index we have of the condition of the latter. Yet in studying the anemias clinically the organs which form the plasma must be most considered; especially the intestine, the liver and the kidneys. It is in the intestinal wall that the plasma obtains its proteid content; in the liver that many processes are brought about including the control of the carbohydrate content of the plasma, the transformation of the ashes of the body to urea, etc.; and it is in the kidneys that the most of the ashes are removed. Certain of the glands of internal secretions also are important in modifying the constitution of the blood, especially the pancreas, thyroid and the adrenal. Lastly, in the muscles themselves the blood is modified since they remove certain tissue constituents and glucose and give back in return the ashes of these bodies.

In many diseases of the blood it is probably the plasma that suffers first, while the changes in the red blood-cells are the results of the plasma changes but also of secondary changes in the blood-building organs.

The ANEMIAS have been classified as *primary* and *secondary*. By PRIMARY ANEMIA was meant one which seems to develop independently of any organic disease, *e. g.*, chlorosis, the essential idiopathic anemias (the simple primary and the pernicious anemia, leukemia and pseudoleukemia). By SECONDARY ANEMIA was meant one for which an adequate cause seems present, as hemorrhage, blood poisons and organic diseases as lues or cancer. The above classification is purely clinical, not hematological, and the autopsy table not infrequently shows to be secondary an anemia which was during life supposed to be primary. Lately the terms primary and secondary relate more to the blood pictures or types rather than to the cause and are being replaced by the terms megaloblastic, chlorotic, etc.

By HYPOPLASTIC OR APLASTIC ANEMIA is meant one due to insufficient blood formation; by CONSUMPTIVE OR HEMOLYTIC ANEMIA, one due in part at least to increased blood destruction.

Secondary Anemia.—A secondary anemia is, from the point of view of the pathologist, one which can be assigned to some cause which would seem adequate to explain the condition. But to the hematologist the term suggests a blood the most of the red cells of which are smaller and of lighter weight than normal, while a few are large, pale and "waterlogged." We usually may make out certain features which have in the past suggested that a toxin has injured the red cells: basophilic granular degeneration, polychromatophilia, etc., but these may just as well be evidence that these cells are immature. The light-weight cells would suggest that the formation of new hemoglobin is a much more difficult function than is the multiplication of new cells (granting that the material is at hand out of which these new cells may be made). In response therefore to an unusual demand on the erythroblastic tissue for new cells the hemoglobin available would seem to be divided into a large number of light-weight cells rather than into a smaller number of normal cells. This we can understand since from the point of view of the physiologist the area of surface of the cells is of more importance than is their mass. As the patient improves the light-weight cells are gradually replaced by those of normal weight, therefore the color index approaches 1.

In mild cases of secondary anemia the count may be normal but the hemoglobin is diminished and the specific gravity slightly lowered since many of the cells are light-weight, *i. e.*, are small and pale. In cases a little more marked (moderate grade) the count may be normal, but the reds are not only light-weight but show qualitative changes: degenerations (?), anisocytosis, poikilocytosis, crenation, polychromatophilia and less tendency to rouleaux formation. In severe cases the blood shows both qualitative and quantitative changes but the count is not much reduced except in the anemias of childhood, after large hemorrhages, in malaria and in acute septicemia. In very severe cases one sees also the evidences of degeneration (?) and destruction (?) of the cells and signs of regeneration (nucleated reds).

BLOOD PICTURE.—In secondary anemia the blood may grossly be pale. The reds are less reduced than is the hemoglobin; their count may even be normal, but in severe cases there will be a great reduction, as in v. Limbeck's case with recovery with a count of 306,000. A reduction of 1,000,000 cells is, Bezançon and Labbé consider, a mild hypocythemia, 1 of from 2,000,000 to 3,000,000 an intense; while if the cells are reduced to 1,000,000 an extreme hypocythemia.

The reduction in hemoglobin is the constant and most important feature and the best index of the grade (yet see page 503) of a secondary anemia. The color-index is low in cases due to cancer, hemorrhage and gangrenous processes, yet not quite so low as it is in chlorosis. On the other hand in cases with extreme oligocythemia the body, if given sufficient time, seems to protect itself by increasing the color-index, that is, by the production of cells which in size or weight are normal or above normal. Some think that the high color-index of pernicious anemia itself is not characteristic of the disease, but a reaction to the low count; the body, because of the chronicity of the disease, having had time to thus protect itself, while in those cases of secondary anemia with low count and low color-index the acute course prevents this protective measure. Another suggestion we would make is that because of abnormal hematopoiesis the bone-marrow, unable to use in new cells all the pigment liberated by the normal breaking down of worn-out cells, saves some of this hemoglobin by overloading the cells it can produce. The specific gravity of the blood is low. The dried residue is reduced. This is especially true in the cancer cases. (In 1 case of cancer of the stomach with a count of 1,400,000 and 15% Hb the dried residue was only 9%.) And yet a lowering of the red cell-count and of the hemoglobin may be a sign of improvement. This is well seen in some cases of anemia with a reduction of the total volume of the blood. During the convalescence in such cases the blood volume is first restored by an increase of plasma which dilutes the blood and gives the appearance of a progressing anemia.

Morphologically the stained cells show a lack of hemoglobin and yet a good many are normal. In many the biconcavity is too evident and pessary forms are common. The polychromatophilic degeneration is common and is seen within 24 hours after a hemorrhage, but bears no relation to the hemoglobin-content of the cell. The number of these basophilic cells runs so parallel to the grade of the anemia that the estimation of their number has been suggested as a substitute for the more difficult blood-counting (Walker).

Poikilocytes occur only in the severest cases. Anisocytosis is marked. Microcytes are always found, some even but 2 μ in diameter. Large cells "acutely dropsical" have been described.

The number of nucleated reds varies much and bears no relation to the anemia, either to its grade or its cause. They may be abundant or absent. Blood crises, normoblastic as a rule, are not rare (see page 606). Microblasts are met with in the severe post-hemorrhagic type and megaloblasts are exceedingly rare except in cases due to malaria and other diseases which affect the bone-marrow.

The number of the leucocytes varies from a leucopenia to a leukemic condition, depending on the cause of the anemia and its complications. During convalescence a moderate leucocytosis with an increase in the polymorphonuclear neutrophiles often develops, due to the increased activity of the bone-marrow. The number of eosinophiles varies much, from few to an extreme eosinophilia. As a rule their count is at the upper limits of normal.

The platelets are increased, even doubled in number. This is always true in the post-hemmorrhagic cases.

Acute Post-Hemorrhagic Anemia.— An anemia due to hemorrhage may be acute or chronic depending on the number of hemorrhages and on the length of the intervals between them. The loss at one time of ½ to ⅔ the volume of blood is fatal. Women tolerate hemorrhages better than men and children least well of all.

Immediately after a hemorrhage the blood is for a while qualitatively normal; then, as the tissue-lymph pours into the vessels to restore the blood volume, the count and the hemoglobin diminish and the specific gravity becomes somewhat lower since tissue lymph is richer in water than is the plasma. The loss of even but 50 to 70 c.c. of blood is followed by demonstrable changes. The color index therefore should for a short time remain "1" then will decrease, since the new cells are "light weight," smaller in size, paler in color than normal and abnormal both in shape and staining qualities. The red cell-count then rises slowly until normal and the hemoglobin more slowly since it is weeks before the light-weight cells can be replaced by new ones of normal volume.

The platelets are increased in number. After 1 hemorrhage the hydremia is maximal and the color-index minimal on about the ninth day. There is often a post-hemorrhagic leucocytosis. The regeneration of the red blood-cells is so rapid at first that some suspect direct division of some of the red blood-cells of the circulation and point out in favor of this the number of small cells and of poikilocytes which appear so early.

One usually finds a few nucleated reds, usually normoblasts, their number related more to the acuteness of the hemorrhage than to its severity, while during convalescence blood crises are common (see page 606).

In 1 case of severe post-hemorrhagic anemia 13.7% of myelocytes were found in the circulation. These disappeared in 3 days. In another very severe case the polymorphonuclears were free from granules.

An early feature in the regeneration of red cells is the production of so many megalocytes that in some cases they are a conspicuous element of the blood-picture.

Time for Regeneration After One Hemorrhage.—The table given by v. Limbeck is:

If the loss of blood was 4.5% of the body weight 30 days are necessary for complete regeneration; if 4% of body weight, 20 days; if 3%, 10 days; and if 2%, 8 days.

Grawitz says a loss of 3 to 4% of body weight requires from 14 to 30 days for regeneration; one of 1 to 3% from 5 to 14 days; a slight loss from 2 to 5 days.

But the time varies also with the age and nutritional condition of the patient, the diet and the therapeutic measures used.

Regeneration is quickest in men between 20 and 40 years of age; slower in women and slowest in children. After the regeneration is complete there may develop even a hypercythemia.

In 1 case due to repeated hemorrhages following abortion (produced evidently by some drug, not by an operation) the count on admission (the temperature was normal) was 1,108,000, hemoglobin 18% and the leucocytes 4625.

In a case of hemorrhage from a badly crushed arm and after infusion the red cells fell during 36 hours from 5,000,000 to 3,000,000, and the hemoglobin from 70 to 50%. The hemoglobin in a case of metrorrhagia fell to 19,% and in another case after 2 post-partum hemorrhages to 11%. Both patients recovered.

Among the causes of acute anemia are: traumatic hemorrhage, tubal pregnancy (in which a rapid anemia is a bad sign), abortion, uterine submucous tumors, ulcers of duodenum and stomach, typhoid ulcers, phthisis, aneurisms, varicose veins of esophagus, rectum, or legs, the "hemorrhagic diatheses" and hemorrhagic pancreatitis.

A case of purpura hemorrhagica of eight weeks' duration [112] was admitted with red cells 696,000, hemoglobin 17% and leucocytes 4000 (s. m. 75%). At death 7 days later the red count was 483,000, there were no poikilocytosis (since too acute?), no nucleated reds and no eosinophiles.

Ewing mentions a case of 3 weeks' duration with repeated epistaxis and a red count of 456,000.

In this group of the acute hemorrhagic anemias are included cases of repeated hemorrhages, yet with hemorrhages at such intervals that complete regeneration after each is possible. In these cases the total amount of blood lost may be enormous. This was well seen in the days when venesection was a common practice. Ehrlich mentions a Russian physician with pulmonary tuberculosis who, in 6½ months, lost 20 kilos of blood (that is, an amount 4 times the total blood volume at any time) and yet recovered perfectly.

Anemia from Chronic Hemorrhage.—By chronic hemorrhage is meant a succession of hemorrhages at such intervals that the patient cannot recover from one before the next occurs. The results are much more serious than those following 1 hemorrhage, or a series with longer intervals, although the total amount of blood lost may be relatively small. A case with repeated epistaxis due to telangiectasis of the nasal mucosa was admitted several times, once with red cells 2,288,000, hemoglobin 18% and leucocytes 2800. Scurvy, especially cases with much hemorrhage, causes a secondary anemia, the red count averaging from 3,000,000 to 4,000,000. In 1 severe case it was reported as low as 370,000. A leucocytosis is often met with in the chronic post-hemorrhagic anemias but is due to some complication, since a leucopenia is the rule. In one case in this clinic the red cells were 2,200,000, hemoglobin 40% and leucocytes 2850. This form of anemia is common in cases of pulmonary tuberculosis, submucous fibromata, hemorrhoids, gastric and intestinal ulcers, cancers (*e. g.*, of the stomach), intestinal parasites, etc. The severe anemias from high and hidden piles is now attracting much attention.

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[112] Billings, Johns Hopkins Hosp. Bull., May, 1894.

As a result of a long-standing anemia the blood-building organs seem to lose their ability to regenerate the blood and convalescence is therefore very slow. In 1 case of hemorrhoids with a count of 2,600,000 it required 8 months to reach normal (Ehrlich). Sometimes a chronic secondary anemia assumes the picture of a primary anemia, sometimes without any signs of regeneration and is rapidly fatal. That these anemias are due in part to the chronic diseases which cause the hemorrhages is one reason why it takes much longer for the blood in these cases to regenerate.

In this form of anemia the hydremia is marked, the specific gravity of the blood is low and the dried residue considerably diminished. The red count is much diminished, even to 1,000,000 cells. The new reds are small and pale and the index low, 0.5 or even 0.44. Nucleated reds are scanty and the platelets are increased. Sometimes the picture becomes that of a pernicious anemia (it may be that other patients die before this blood picture can develop), yet in fatal cases the index usually falls progressively until death. The leucocytes often are increased at first but when the anemia becomes very severe a leucopenia usually exists.

BLOOD POISONS.—Certain poisons may cause anemia by destroying the corpuscles themselves or by injuring the blood-building tissues. Among them are the toxins of the infectious diseases, especially the septicemias, scarlet fever and lues; certain mineral poisons, as lead, arsenic and mercury; the toxins of intestinal parasites, as Bothriocephalus latus; and especially the toxin of malignant tumors.

The effect of these poisons is sometimes seen in various degenerations(?) of the red blood-cells in the circulation and in the hemoglobinemia (plasmolysis). Other toxins are thought not to injure the cells in the circulation but to cause an increased activity on the part of the blood-destroying organs, the liver, the spleen and the marrow. The best illustration of the effects of such a hypothetical toxin is hematochromatosis. It may be that the poison produces a chemical (plasmotropic) change in the protoplasm of the red cells which singles them out for destruction.

ANEMIA OF INANITION; THE ANEMIA OF THE POOR.—The anemia seen so often among the poor is considered by some as a simple primary anemia, by others as a secondary anemia due to a variety of concurring factors the relative importance of which cannot be apportioned, such as poor food, lack of sunlight, bad air, worry and overwork. The present idea is that it is due to long-standing latent infection.

Starvation alone will not cause an anemia; that is, it will not produce qualitative changes in the blood but causes rather a diminution in the total volume of blood which runs roughly parallel to the loss of weight. The blood of Cetti, at the end of a 10-day fast, showed a rise in the red blood-cell count of 1,000,000, a slight fall in the hemoglobin, while the leucocytes fell from 12,000 to 4200. In such cases the blood picture of anemia may begin with the improvement of the patient's condition after he begins to eat since the formation of new blood is a slower process than the gain of the other tissues. The first blood changes as these patients improve will be those of dilution, *i. e.*, an apparent anemia.

Poor food has been considered an important cause of chronic anemia of the hypoplastic form (Immermann), *i. e.*, of anemia due to insufficient blood formation. In support of this view it is emphasized that the foods which contain most iron are the most expensive and that anemia is met with particularly in those European countries where the diet of the poor consists chiefly of bread, potatoes, etc. And yet this cause is greatly exaggerated. In this country at least the trouble is not so much the quality of the food, since even the poorest contain enough of this metal to replace that which daily is actually lost to the body, as its preparation, good meat and vegetables being rendered indigestible in the frying-pan; and also the American habit of eating in a hurry and insufficiently masticating the food, all of which would cause gastro-intestinal troubles which might result in anemia. Bunge's experiments showed that a diet poor in iron can cause anemia in a growing child, but the problem of growth is a different one. The effect on the blood of a non-proteid diet, however, can be demonstrated at the end of 6 or 8 days, at first by a slight hydremia and later by the changes in the red blood-cells characteristic of a secondary anemia.

Those living in dark rooms are very apt to be anemic, not because of the *lack of sunlight* but because of the prevalence of tuberculosis, an anemia-producing disease common among those seldom in the sunshine. Hemoglobin is not comparable to chlorophyll as the illustrations cited by Ehrlich show. The horses which were kept at the bottom of mines in Germany for from 10 to 24 years without seeing sunlight had normal blood; the members of Nansen's Polar Expedition remained for 140 to 150 days without sunlight and yet remained healthy provided the other causes of anemia were eliminated. On the other hand although sunlight may not be very important for the adult it is for the growing organism (Schonenberger), but other factors also are potent to explain this.

Life in an atmosphere of *bad air* also would seem to predispose one to anemia, while *overwork* and *worry* would superficially, at least, seem important causes of the anemia of the poor. Many so-called "scientific facts" which have been useful in the propaganda of a reform are held tenaciously by reformers who will continue for a long time probably to assign to the above factors the anemia of the poor. And yet indirectly these are its causes and their correction would do much to eliminate this form of anemia and also the anemia of the rich since they lower the resistance of the patients to the various latent infections of lungs, kidneys, teeth, tonsils and bowel and it is these which cause anemia.

There is a group of SIMPLE SECONDARY ANEMIA for which no one cause can be assigned. The great majority are met with in women. In one series the mean of the red cell-count was about 3,000,000 (2,100,000 to 3,900,000), the hemoglobin from 30 to 50% and the leucocyte count about normal. Such cases improve rapidly under treatment.

GASTRO-INTESTINAL DISTURBANCES are important causes of secondary anemia, and possibly also of many so-called primary anemias in patients whose intestinal features were overlooked. The intestinal wall is one of the most important of the hematopoietic organs since it is the source of supplies for the plasma, hence indirectly for the cells.

In 60% of our cases of *severe diarrhea* in men the red count was not above 4,000,000; in women it was a little higher. The real anemia must have been more pronounced than this for in cases of diarrhea the blood is concentrated by the loss of fluid (in one case the count was 7,900,000). The leucocytes often ran low (even to 2700 and 2500) but in 30% of the cases the count was above 10,000.

Cabot mentions 1 such case with 1,928,000 reds, and another with 2,440,000 and 10% hemoglobin

In *chronic dysentery* the red cell-count may be high or low. In 1 case it was 1,520,000, in another 2,500,000. On the other hand, in 1 (a male) it was 7,000,000 with 110% hemoglobin and 7000 leucocytes; and in another (a woman) it was 6,300,000.

In *chronic constipation* our cases showed both normal and high counts.

Our cases of *benign dilated stomach* showed nothing abnormal as regards the leucocytes. The red counts were low yet within normal limits, except in 4 cases which showed considerable anemia (3,300,000, 2,400,000, 2,250,000 and 2,600,000). The vomiting of large amounts of fluid does not seem necessarily to concentrate the blood.

Acute gastritis, during the febrile period, causes a slight leucocytosis and this was true also of 70% of our cases of *gastro-enteritis*. A slight leucocytosis is common also in *chronic gastritis*, except the alcoholic form in which cases the counts were quite low.

In *ulcerative colitis* the red cell-counts below 3,000,000 are not rare.

In *amebic dysentery* a severe anemia is rare, yet in 24% of our cases there was a slight (4,000,000 to 4,500,000) and in 12% a more severe (2,200,000 to 4,000,000) anemia. A leucocytosis was the rule (in 70% of cases) at some time during the disease, the highest count being 19,200. Futcher[113] found the general average of 43 cases about 10,000. In children Amberg found an eosinophilia.

ANEMIA OF THE TROPICS. — It is said that Europeans after a stay of some duration in the Tropics look anemic. Some consider this only apparent although the frequent presence of basophile granulations in the red blood-cells would seem to indicate some injury to these cells. There are several tropical diseases, important causes of anemia, which only now we are beginning to understand and these may explain some of the above cases.

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[113] Jour. Am. Med. Assoc., August 22, 1903.

CHRONIC INFECTIOUS DISEASES.—Of the chronic infectious diseases three are most potent causes of anemia,—lues, tuberculosis and leprosy. While the toxins of the organisms of these diseases may be important yet the gastro-intestinal conditions, the lack of exercise and the frequent hemorrhages so common in these diseases also must be considered.

There is a great difference in the effect of bacterial toxins on the blood. In *acute miliary tuberculosis* without cyanosis, for example, 1 of the worst of septicemias, there is little trace of blood destruction (see page 637), while anemia is a common result of *latent pyogenic infections.* The same is true in diseases with *chronic exudate formation. Albuminuria* is frequently cited as an important cause of anemia and yet the anemia must be due to the cause of the albuminuria for the actual daily loss of proteid to the blood-plasma even in a severe case is very slight.

Spermatorrhea, *lactorrhea*, and *diseases of the respiratory organs* with a large amount of sputum are given as causes of anemia, and yet patients with long-standing profuse purulent exudate formation, as chronic bronchitis and tuberculous abscess of its joints, maintain their blood condition surprisingly well.

In cases with *marasmus* an atrophy of the total blood volume may cover an anemia, while in other cases the anemia may be more apparent than real, since because of improvement the plasma is diluted.

Fever is cited as an important cause of anemia and yet it is not the elevated temperature but the toxins which cause the rise of temperature that destroy the red cells. Most important are those cases of *chronic cryptic septicemia* which for weeks may present the picture of a severe anemia. *Acute infections* may cause a rapid fall in the blood-count, as for instance Grawitz's case of streptococcus septicemia, in which in a little over 1 day the reds fell from normal to 300,000.

A recent case of *arthritis* of unknown cause, but with blood-cultures negative, had a red cell-count of 976,000, Hb 17%, and leucocytes, 4600. He improved rapidly.

In *yellow fever* considerable anemia may develop. In 1 case the count was 2,604,000 and in another 1,400,500 (Maurel).

Pneumonia, *diphtheria*, *scarlet fever*, *typhoid fever*, *acute articular rheumatism*, *smallpox*, *septicemia* and other acute infectious diseases may cause a severe anemia. The reader is referred to the various sections on these diseases. In all such cases there may, for the first few days at least, be no diminution in the red blood-count, but rather a hypercythemia due to concentration of the blood. This is best seen in diphtheria and typhoid fever and it may cover a real anemia. The rapid fall in the count in cases of pneumonia during convalescence or at the time of the crisis, is probably more apparent than real and due to dilution of the blood resulting from a temporary general vasomotor relaxation (Grawitz).

In many cases of infection there is merely a drop in the count, but in very severe cases microcytes, macrocytes or poikilocytes are present. In these latter cases hydremia is the rule, the loss of albumin running parallel to the severity of the disease, and in severe cases reaching even 6.25 gms. of residue per 100 c.c. of blood.

INTESTINAL PARASITES.—Of the intestinal parasites 2 are very important causes of anemia.

Uncinaria Duodenalis et Americana.—Historically this form of anemia is most interesting since the miners' and tunnel diggers' anemia due to this parasite were the first forms of anemia accurately to be described. This was then rated as primary pernicious anemia.

These parasites are met with in many different countries and in this they would seem to be the chief cause of the "anemia of the South." One severe case we saw had a red cell count of 2,424,000, Hb 32% and leucocytes 9700, of which the eosinophiles were 5.6%. Counts below 1,000,000 cells have been reported.

This anemia resembles one due to hemorrhage rather than to a toxin. (Note the small amount of iron in the liver, even but 1/4 the normal amount, the absence of a leucocytosis, and the very low color-index.)

In 3 cases of *Strongyloides intestinalis* infection the blood showed: in the first, red cells 5,420,000, Hb 82% and leucocytes, 6200; in the second, 3,560,000, 57% and 21,500; and in the third, Hb 60% and leucocytes, 7500.

Bothriocephalus Latus.—Bothriocephalus latus (see page 417) may cause a most interesting and severe anemia, the almost exact picture, both quantitatively and qualitatively, of the primary pernicious type, but which will disappear soon after the worm has been expelled. In Lichtheim's case the count of red blood-corpuscles dropped to 500,000 and the hemoglobin to 20%. Six worms were expelled. In Schapiro's case the count was 837,000 and in 23 days after the worm was expelled, 2,975,000. Bezançon and Labbé give 1,300,000 as the average of reds in a series of cases, (the limits were from 395,000 to 2,150,000) while the color-index varied from 0.9 to 1.62. All the signs of a severe primary anemia, the poikilocytes, microcytes, macrocytes, the polychromatophilic degeneration, etc., are present. Even 1/2 of the nucleated reds may be megaloblasts and yet in 2 weeks after the worm has been expelled the megaloblasts may all disappear, in 3 weeks all the megalocytes and soon the blood appears quite normal. The leucocytes are normal both quantitatively and qualitatively.

The cause for this anemia is unknown. It is not the mere presence of the worm, since but 16% of the persons who harbor this parasite have any anemia. It is not the duration of the infection, for some persons are hosts for even 20 years before the anemia begins. There is no evidence of hemorrhage and the amount of iron of the liver has been found even twice normal, which would indicate an intravascular destruction of the red blood-cells. Schaumann believes that the patients must have some predisposition to an anemia. Dehio says that the worm itself must become diseased or die to affect the host's blood. But in some cases with anemia the worm does not appear diseased and in others who harbor a certainly diseased worm there is no anemia, while in still others the anemia persists after the worm has been expelled.

Tænia saginata and *Tænia solium*, *Strongyloides intestinalis* (in cases of the "diarrhea of Cochin China" due to this parasite, counts as low as 760,000 have been reported) and *Ascaris lumbricoides*, sometimes, it is claimed, cause anemia.

YEASTS.—In four cases of *systemic blastomycosis* a moderate secondary anemia has been reported (*e.g.*, 3,992,000 red cells per c.mm.), and in 8 cases the leucocytes varied from 9,600 to 21,200. In 2 of these cases this yeast was isolated in pure culture from the blood.[114] In 1 case of infection with *Oidium coccidioides* there was a leucocytosis of 17,000.[115]

POISONS.—Lead, mercury, arsenic, certain organic poisons, plant and animal toxins, ptomaines and the toxins of burns, all may produce an anemia. *Lead* is a very important cause of both the acute and chronic forms of anemia. It causes essentially a chlorotic anemia, manifested first by degeneration (?) of the red blood-cells, not by any diminution in number, after which the count is reduced even to 1,300,000. Megaloblasts sometimes are found. The basophile granules of the red cells are early very common and important in diagnosis (see page 479).

In the Johns Hopkins clinic there were 17 cases of anemia due to lead. Of 16, the lowest red count was 2,900,000; in 7 the red count was over 4,500,000; the mean was 4,200,000. The lowest hemoglobin was 38%; the mean, 60%. In 10 of 16 cases the leucocytes were above 10,000, with the maximum 25,000. This count fell very soon after admission.

Long-continued use of certain of the *coal-tar products* will cause a severe anemia. Stengel and White[116] report a most interesting case due to acetanilid in a woman whose red cell count was 2,092,000, Hb 35%, and leucocytes 19,800 (a previous count). There were 32,323 nucleated reds per cubic millimeter, of which 91.4% were normoblasts, 3.5% megaloblasts and 5.3% free nuclei. The number of platelets was increased. Many of the red cells were poikilocytes, a few contained basophile granules and many showed polychromatophilic degeneration. The diagnosis in this case was suggested by the appearance of the smear alone. They mention Ehrlich and Lindenthal's case in which 1 of each of 56 red cells was nucleated. In Brown's case of acetanilid poisoning[117] the reds at death numbered 1,166,000, of which 22,150 per c.mm. were nucleated.

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[114] Montgomery and Ormsby, Arch. of Int. Med., Aug., 1908, vol. ii, No. 1, p. 1.
[115] Hektoen, Jour. of A. M. A., Sept. 28, 1907, vol. xlix, p. 1071.
[116] Contrib. of the Wm. Pepper Laboratory of Clinical Medicine, 1903, No. 4.
[117] Amer. Jour. Med. Sci., 1901, vol. cxxi.

Splenic anemia is the name given to a group of cases of anemia of the secondary type associated with idiopathic enlargement of the spleen. The count in Osler's cases averaged over 3,000,000. The leucocyte count was normal or reduced. Such cases suffer profuse hemorrhage from gastric and esophageal varices. In 1 of Osler's cases the macrocytes and gigantoblasts were a marked feature.[118]

Simple Primary Anemia.—Some authors attempt to separate a simple primary anemia from the primary pernicious form, basing their differences on the clinical course which is characterized by the number of relapses and which ends finally in death. It would be hard to separate this disease from the secondary anemias already mentioned as due to bad unhygienic conditions. These cases seem to stand midway between chlorosis and primary pernicious anemia, with oligocythemia and oligochromemia of about equal grade and with leucocytes normal both quantitatively and qualitatively.

Progressive Pernicious Anemia.—Eichorst's definition of primary pernicious anemia is, a severe anemia which in spite of all treatment progresses relentlessly to death. Many cases, however, live for years with occasional remissions and intermissions. It is usually referred to as a *hemolytic* anemia. Clinically the blood picture is very definite although not characteristic. Pathologically there is evidence of an excess of broken down hemoglobin and bone marrow lesions which are almost characteristic; that is, a hyperplasia of red marrow containing an unusual number of megaloblasts. The blood picture alone is not characteristic, for many cases of definitely secondary anemia have a similar picture, *e. g.*, the "secondary pernicious anemia" which develops in rare cases of cancer, phthisis, lues, malaria, following repeated hemorrhage, lead poisoning, certain animal parasites, lesions of the bone-marrow, especially tumors, also osteomyelitis, atrophy of the gastric mucosa. stenosis of the pylorus, nephritis, certain rare cases of pregnancy and purpura hemorrhagica. Many of these cases give a history of anemia-producing conditions which for years may have led to almost complete bankruptcy of the blood-building function. The only difference between these cases and primary pernicious anemia is the absence in the latter at autopsy of any of the lesions of these diseases.

The salient characteristics of the blood in primary pernicious anemia are: A megalocytic blood picture including megaloblasts; anisocytosis; poikilocytosis; a high color-index (because of the megalocytes); marked evidences of blood regeneration (nucleated red cells of all types); and signs which have been interpreted as those of rapid blood destruction (degenerated (?) reds, endoglobular degenerations, polychromatophilia, urobilinuria, jaundice, the increased iron compounds (?) in the serum and the corpuseles, and the increase of iron stored in the liver and spleen). The student should remember that the presence of all this pigment need not indicate increased hemolysis but the effort of the body to get rid of hemoglobin which it can no longer use. There is a constant and normal breaking down of cells which have served their purpose (at least 18 gms. of hemoglobin per day and probably more) and if the bone-marrow is unable because of definite disease to build new cells fast enough some of this pigment must be destroyed. At first the poikilocytosis was supposed to be characteristic of this disease (Quincke) but this idea was very soon corrected; then the high color-index (Laache and Kahler) and this is still the opinion of many; then the presence of megaloblasts (Erhlich) but this is not true, although they are particularly numerous in these conditions. The diagnosis cannot be made from any one feature.

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[118] Osler, Am. Jour. Med. Sci., January, 1900.

Volume of the Blood.—There is no satisfactory clinical method of determining blood-volume but in pernicious anemia the appearance of the patient suggests and later the autopsy shows a great reduction in the amount of blood in the heart and blood-vessels. In a remarkable case seen in Professor Müller's clinic all the organs seemed almost exsanguine.

Gross Appearances.—The ear is a better place to obtain a drop of blood than is the finger. It may flow freely or not at all. Lazarus considers that the former occurs when the patient is doing badly and that the latter is evidence of improvement. The blood is pale, of a light red, watery color and may not at all resemble blood.

We held up a tube full of this blood before a class on one occasion and asked them to tell from its appearance alone what fluid it was. Many of them said it was a cloudy urine, which, indeed, it did resemble.

The drop of blood is often grossly streaked, evidence that the corpuscles have collected in masses. Cases have been described in which it grossly is of a coffee-color, probably due to hemoglobinemia. The coagulation time is often increased.

Red Blood-cells.—In the fresh specimen the poverty of the blood in red corpuscles and the absence of rouleaux formation are conspicuous features. The cells vary much in size (anisocytosis); the majority are slightly above normal, some are very large and others are small, some very small. A few show Maragliano's endoglobular degeneration and a few more show another degeneration, the accumulation of the hemoglobin in the center of the cell, but the most have a uniform dark color. Nucleated reds often are numerous. In a well-marked case the appearance of the fresh specimen alone will strongly suggest the correct diagnosis.

An extreme oligocythemia is the rule in pernicious anemia. It is remarkable how few symptoms the patients with these low counts suffer, particularly as the volume of blood also is dimished. On the first visit the average cases will show a count of about 1,000,000 cells. Cabot's average was 1,200,000.

In the 81 cases of the Johns Hopkins series which we studied the average red cell-count on admission was 1,575,000. This is somewhat higher than that which other observers have reported, since we included in the series patients admitted for complications of the disease and not for the asthenia of the anemia alone. In 81% of these cases the count on admission was under 2,000,000 and in 12% under 1,000,000.

Some patients with counts as low as 500,000 are comfortable and active, while others with 4 times that number of cells suffer severely. Evidently the reason for their symptoms is not the oligocythemia alone and yet the first counts which the same patient has on several successive admissions are often curiously similar. The count may remain stationary for some time, often in the neighborhood of 1,000,000, or it may diminish progressively until death. Quincke reported 1 case with a blood-count of 143,000 who recovered. Hayem reported a fatal case whose lowest count was 292,000. Scott's case at death had 268,000 red cells, a color-index of 2 and 5900 leucocytes per c.mm. [119]

After treatment begins the count may continue to drop for a while and then rises; or it begins to rise at once. In a few cases it remains stationary, but as a rule there is a tidal rise and fall of the count which is important in diagnosis.

It cannot too often be emphasized that a change in count may mean a change in the total number of red cells or a change in the volume of plasma and that improvement is usually ushered in by a drop in the count due to an increase in the volume of plasma.

It is interesting how few clinical symptoms seem related to the red blood cell-count. Judged by the comfort of the patient alone a case of pernicious anemia with a count of 1,000,000 is more comfortable than a case of chlorosis with a count of 4,000,000. Again, in some of those cases in which the count continues to fall after admission and then later rises, it is curious that the patient feels so well that he insists upon going home at a time when the count is no higher or very little higher than on his admission. In other cases in which the count rises after admission and then falls, death occurs when the count has reached about the level of admission. In still other cases with an initial drop, as in 5 of our series, the count was rising at the time of death.

The red blood-count on the day of death of 2 of our cases was high—2,700,000; in 3 cases it was moderate, 1,031,000, 1,326,000 and 1,216,000; while in 13 cases, and this, we think, is a hint of the blood picture if death is due to this anemia alone, the count was between 718,400 and 376,000, an average of 567,700.

The blood during the intermissions of this disease is not quite as normal as the subjective feelings of the patients would suggest. The red count averages about 3,000,000, the cells are still a little large (Cabot), the nucleated cells have disappeared, the color-index is usually high (but sometimes low) and the leucocyte count is increased by an increase in the polymorphonuclears. The diagnosis of this disease during intermissions is important, especially to insurance examiners. Two of our cases with almost normal blood (1 was refused by 1 examiner) both succeeded finally in getting heavy insurance and died in about 1 year.

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[119] Am. Jour. Med. Sci., 1903, vol. cxxv., p. 397.

The *volume* of the red blood-cells, which is determined better by sedimentation than by centrifugalization, averages from 8 to 10%, which is high relative to the count and is a measure of the large size of the cells. Capps found that in this disease the volume index is always higher than the color-index. The cells are little biconcave (seldom does one see pessary cells) but seem plump although shadows do occur.

The average diameter of the red cells in primary pernicious anemia is definitely increased, although the variations in size are very wide, in general from 4 to 13μ, but with extremes beyond these. In no other disease are there so many macrocytes and also microcytes but the mean size is definitely increased (a few cases are mentioned in which the blood is not megalocytic).

Seventy per cent. of the cells are *macrocytes* and measure between 11 and 13μ in diameter (Lazarus). In a case reported by Ewing 90% measured from 11 to 16μ. Gigantocytes are numerous. These huge cells are not biconcave, sometimes are biconvex, often are oval and seem flabby. Many appear dark in fresh and polychromatophilic in stained specimens. A few may be pale (Grawitz) ("chlorotic cells," "dropsical cells"), but the dark color of most of them is a quite constant feature. Some cells seem to have a slight change of color shade as well as of color depth. The presence of macrocytes is considered (Laache) a compensatory attempt to replace the amount of hemoglobin-containing protoplasm; Cohnheim first said it was reversion to the embryonic type; Ehrlich attributes it to a megaloblastic degeneration of the bone-marrow (see page 502).

Microcytes, cells which vary from 2 to 6μ in diameter, occur in large numbers here as well as in the secondary anemias. So numerous may they be that the average size of the red blood-cells may not be above normal. Their dark color may be due to their spherical shape, but they have sometimes a greenish tint, suggesting a chemical change in the protoplasm. These microcytes change their shape definitely; they also move quite actively among the other cells with an oscillatory motion which suggests ameboid activity. They have been described as monads, a leptothrix form, bacteria, and Hayem called them pseudo-parasites (see Fig 117).

Poikilocytes, or deformed cells, are present in great numbers and in a great variety of shapes: hooks, raquette forms, spindle and various dwarf forms (microcytes, see above), but it is the sausage and battledore shapes which, it is claimed, are found here only. Since in an acute case abnormal cells did not appear until about 2 months from onset, McCrae suggests that such cells are products not of degeneration but of defective regeneration.

Polychromatic "degeneration" is best studied in pernicious anemia. Despite the name many of these cells may be young forms. With Ehrlich's triple stain they are a pale gray (Plate I, 25-28), while with methylene blue they take a blue tint. Their number seems almost parallel to the severity of the case (Grawitz). Red cells with Grawitz's basophile granules are very common, especially in severe cases, and have, Grawitz thinks, an important diagnostic value.

Nucleated Reds.—*Normoblasts* (Plate I, 29, 30, 35, and Fig. 124, *a*, *c*, *d*, *e*), described on page 500, occur quite constantly in pernicious anemia, alone or with *megaloblasts*, and in especially large numbers during the blood crises. In a case of Bezançon and Labbé there were from 6000 to 10,000 normoblasts and 960 megaloblasts per cubic millimeter. Many of these cells show polychromatophilic degeneration, especially those in which the nucleus is dividing.

The *blood crisis*, so interesting a feature in cases of severe anemia (see pages 501 and 608), is not always, as v. Noorden thought, (except in young patients), the sign of a regeneration active enough to be followed by a rise in the red count, although in secondary anemia and chlorosis this usually is the case. The crises probably indicate an attempt of the bone-marrow to replenish the blood, but these attempts often are futile and suggest a convulsive attempt to stem the tide of the anemia.

In some cases there are few or no nucleated reds in the peripheral blood. This means a slower regeneration. In other cases just before death all these nucleated cells disappear.

Megaloblasts (Plate I, 32, 33, 38, and Fig. 124, *f*) were first described by Ehrlich as characteristic of pernicious anemia. They may, however, occur in any form of anemia and in any disease which involves the bone-marrow. Since there has been little agreement as to the definition of a megaloblast the literature on this subject is much confused. To some a megaloblast is any large nucleated red; to others it is a red cell with a large nucleus; while others describe it as a red cell with a characteristic nucleus irrespective of the size of the cell. In the blood of these cases one finds large red cells with nuclei like those of normoblasts and smaller cells with nuclei like those of megaloblasts. We count both these as intermediate cells, and reserve the term megaloblast for a large red cell whose nucleus is at least the size of a normal red blood-cell; that is, 7 μ in diameter. These cells are round or oval and vary from about 11 to 20 μ in diameter. The very large ones are called *gigantoblasts*. They are plump, often diffluent, stippled and polychromatophilic. The nucleus is plump, round or oval and often surrounded by a clear circle outside of which the protoplasm often stains deepest; karyokinetic figures are sometimes seen which to some is a grave sign. In the fresh specimen these nuclei have a well-defined chromatic network, but often stains so faintly that it may be overlooked. The fresh unstained specimens should be studied whenever possible since it is no easy matter to tell in the stained specimen a polychromatophilic megaloblast with a palely staining nucleus from a mononuclear leucocyte; indeed it may be impossible (Plate I, 36). Color, in a stained specimen, counts but little for there is no staining reaction which is characteristic of hemoglobin especially when basophilic. It is of assistance, however, that the protoplasm of the red cell is more opaque, that its margin is thick, smooth, uniform and rounded (best seen when this cell touches another), while the spherical leucocyte which has flattened out in the preparation has a thin, frayed margin.

While megaloblasts may be found in any anemia they are quite common in this and yet they may be hard to find. Ehrlich, it is said, would hunt for hours until he found this much-desired cell upon which he would base his diagnosis. In some cases for a while none will be found and later, many. Their presence in large numbers is an ominous sign. They are sometimes present during the periods of intermission while the blood-count is almost normal.

Typical megaloblasts are found most frequently in pernicious anemia, even mild cases, and for the diagnosis of this condition in adults they are of great value. In the various anemias of children, however, one frequently meets with them. In adults they may be numerous in splenomyelogenous leukemia, in bothriocephalus anemia, in malignant disease of the bone-marrow and in malaria, especially of children, even when there is no marked anemia; that is, in those conditions in which the bone-marrow is involved. Their presence may be an indication of the severity of an anemia, of its form, or of bone-marrow involvement. Some have described them as swollen, hydremic cells which contain the increased water contents of the plasma. If so they would be similar to the so-called "chlorotic cells" seen in cases of marked hydremia, as nephritis, but also in chlorosis, in which disease the plasma is practically normal. But these they do not at all resemble.

Intermediate Forms (Plate I, 31, 37, and Fig. 124, *b*) are those nucleated red cells too large, and with a nucleus too large, to be called normoblasts and yet not typically megaloblasts.

That such cells could be transitional between megaloblasts and normoblasts was denied by Ehrlich and Pappenheim but claimed by others (Schaumann). They are seen only in those conditions in which megaloblasts would be expected, and have, we believe, practically the same significance (see page 501).

Microblasts are cells with a nucleus like that of normoblasts, although usually more pycnotic, but with protoplasm exceedingly scanty and often ragged on the margin. Whether these cells are normoblasts which have undergone some degeneration or are a definite type of cell is not yet known. They are met with in pernicious anemia but also in severe secondary, especially the post-hemorrhagic, anemias.

The presence of nucleated reds was noted in 57 of 69 of the Johns Hopkins series of cases of pernicious anemia. In 13 definite blood crises were present; that is, there were more than 50 nucleated reds per 1000 leucocytes. This is rather an arbitrary line, and yet we have found that, in these cases at least, it corresponded quite well with the blood pictures. In all cases some of these cells were normoblasts while in 40 (58%) megaloblasts were also present. In the other 6 cases the nucleated reds were normoblasts and intermediate forms.

During the course of these 57 cases were 63 periods with nucleated reds present. Of these 26 (that is, 41%) were followed by a gain in the red cell-count; the rest by either no gain or by a loss. Of 14 periods with nucleated reds absent, 8 were followed by a distinct gain.

In 13 of these cases (19%) blood crises developed. Five of these cases died. Of the 50 or more nucleated cells per 1000 leucocytes which constituted the crises, the normoblasts varied in number from 5 to 3128; the intermediates reached even 212 and the megaloblasts 44. There seem to be 2 definite forms of blood crises; those in which normoblasts largely predominate and those in which the intermediate and megaloblasts also are present in considerable numbers. The normoblastic crises particularly are followed by a rise in the red count; those with many megaloblasts present would seem to be less efficient and to appear especially when the patient is losing ground.

The most remarkable blood crisis of our series lasted for 19 weeks, during which time the red blood-cells, at the beginning 1,902,000, rose to 2,562,000, and then at death had dropped to 1,328,000. The leucocytes, meanwhile, varied from 3000 to 5000 until the day of death when the count was 16,000. During this whole period the blood contained over 500 normoblasts per thousand of leucocytes, on one occasion 1164, a little later 1032, and once 3128. Since on this last day the leucocyte count was 4600 the total number of normoblasts must have been 14,388, the intermediate forms 460 and the megaloblasts 138 per cubic millimeter.

HEMOGLOBIN.—The hemoglobin is much reduced in pernicious anemia. It is rarely above 50% and often is as low as 10%. The color-index, however, is normal or high, a point of great importance in diagnosis. In our cases the hemoglobin on admission averaged 34% and the color-index 1.1. In 80% of the cases, it was over 1 and in 2 cases as high as 1.9.(!)

Ewing considers that a low index indicates a chronic case and a high index an acute one. For it to rise is considered a bad sign since it indicates a falling count; with improvement there is always a lowering of the index since so many of the newly formed cells are of lighter weight than normal.

The high color-index in pernicious anemia has received various explanations. One is that it is the result of abnormal "globular richness"; that is, of an abnormally large amount of hemoglobin per cell. This idea is confirmed by estimations made of the weight of the cells and by Capps who found that the color-index never exceeds the volume-index. Another explanation is that it is due to the large number of macrocytes present and indeed the hemoglobin curve does run fairly parallel to that of the number of these large cells. Others ascribe it, and with good reason, to incorrect blood counts in which a great many of the microcytes have been overlooked, while their hemoglobin does add to the color test. Others say that in pernicious anemia some of the hemoglobin is free in the plasma. Some believe that the chemical composition of the red blood-cells in this disease is not normal and find their nitrogen increased (v. Jaksch), hence the name "hyperalbuminemia rubra." Others find more iron present than theoretically the hemoglobin molecule should contain, which would mean either that iron is increased in the hemoglobin molecule, or is present in other combination. In favor of this is the hematogenous jaundice so often present and the discovery reported of iron compounds in the plasma. Taylor considers the high color-index an optical illusion; Grawitz (and this view appeals to us very strongly) warns against hemoglobin determinations with an ordinary hemoglobinometer and emphasizes the danger of overlooking microcytes in blood-counting. He considers that the index is best determined by the appearance of the cells and thinks that the inequality in the distribution of protoplasm and the production of poor cells are the prominent features of pernicious anemia. Bezançon and Labbé say that the appearance of the cells does not suggest that they are overrich in hemoglobin. It is our opinion that it does (see page 501). That changes in the composition of hemoglobin occur and can make red blood-cells appear darker than normal is illustrated by many degenerating cells, by cells picked up by phagocytes and by the brassy cells of malarial blood.

It is important to remember that the hemoglobinometers with a color prism do not, as bought, give accurate readings in the lower half of the scale unless they have been especially standardized and that an error of 5%, so insignificant in normal blood, changes the index considerably when added to a total of but 10%.

During the course of our cases the hemoglobin, as a rule, ran parallel with the count of red blood-cells. As the cases became worse the index slowly rose and at death averaged 1.5. This may have been due to the tendency of the marrow to form large cells.

Leucocytes.—In severe and uncomplicated cases of pernicious anemia there is always a leucopenia. Cabot's average count was 3800 and in 72 of the 110 cases it was below 5000. The leucocytes in our cases on admission averaged 4600. This includes all cases, even those with leucocytoses due to complications. In 75% of these cases the count was under 5000. In some it went as low as 2000 or 1500 and before death even to 500 cells per cubic millimeter. The leucocyte count runs, as a rule, parallel to that of the red blood-cells. A leucocytosis may mean a complication, as pneumonia, a pyogenic infection or a blood crisis, in which case the large number of leucocytes may even suggest leukemia. At death, the picture may be leukemic (100,000).

The leucocyte count in our cases ran fairly parallel to the red count. At death it varied from 660 to 16,000 and averaged 5950.

Of our 81 cases, in 55 (70%) this count fell at some time during their stay below 3000; in 32 (40%) below 2000; in 9 (11%) it fell to 1000 or below. These very low counts, 1000 or below, are found only in the severest cases.

The percentage of polymorphonuclear neutrophile cells runs roughly parallel to the total leucocyte count. This is well illustrated by the rise of these cells with the improvement of the case and their low percentage when the count is low.

The absolute number of the mononuclear non-granular cells tends to be quite constant, therefore their percentage will vary inversely with that of the granular cells. The highest per cent. in our cases was 93%. It is also true that in any given case the percentage relation of the various types of leucocytes tends to be constant whatever the total count of these cells, which indicates that some of these variations are due more to the distribution of cells or to dilution of the blood, perhaps from stasis in the vessels, than to any real change in the blood formula.

Toward death the percentage of the mononuclear non-granular cells rises, probably because the granular cells are then being formed in diminishing numbers. It is interesting that the variations in the percentage of these cells show definite waves during the course of the disease.

The percentage of lymphocytes is always high, averaging 45%. This seldom indicates a true lymphocytosis but rather an absolute decrease in the number of polymorphonuclear cells. Their percentage may reach as high as 62 and before death even 79%, and yet their absolute number remain normal. This has been given as evidence that these cells arise in the lymph-glands and not in the bone-marrow. This decrease in the number of polymorphonuclear cells is a striking feature in pernicious anemia and in diagnosis is used to exclude cancer and septic anemia. Yet even in the same case these cells vary so much that this point is not of great importance.

Twelve (17%) counts indicated a true lymphocytosis which was temporary in every case. In one there was a definite leucocytosis, while in the others the total counts were not above normal limits.

The eosinophiles averaged about 2.7%. In 1 case they reached 9%. They are often absolutely increased, but in severe cases are more often diminished.

Myelocytes are often found. In no disease except leukemia are they so constantly present or their number so great as in pernicious anemia. In acute exacerbations of this disease they may make up even 29.4% of a total of 34,000 cells (Billings). Eosinophilic myelocytes sometimes, but rarely, are met with.

In 23 of our cases myelocytes were present in numbers varying from 0.2 to 8%. Nine of these cases were fatal. In 12 cases the percentage was not above 1%. In 6 it was above 3%.

In our cases the myelocytes were conspicuous under 2 conditions; in cases with a very low count and when a leucocytosis was present. In 1 case the total leucocyte count was 1800, 8% of which were myelocytes. In another case with 14,400 leucocytes 2% were myelocytes. In another case the leucocyte count was 11,600 of which 0.5% were myelocytes.

Mastzellen were noted in 29 of our 69 cases. In 2 they made up over 3% of the total count and in 8 over 1%. The average total leucocyte count in these 8 cases was 3900. Of the other 21 cases the average per cent. of Mastzellen was 0.5%. If in these cases the cells thus designated really were Mastzellen, then the blood in pernicious anemia shows a definite increase of these cells hitherto not mentioned.

Many of the leucocytes in pernicious anemia show evidence of degeneration; that is, are pale, swollen and vacuolated and their nuclei fibrillar. The neutrophile granules of some cells are crowded at the periphery. Hayem considers that some imbibe a certain amount of hemoglobin.

Some cases of pernicious anemia resemble closely acute leukemia. In 1, for instance (Williamson and Martin), the red blood-cell count was 300,000, the hemoglobin 12% and the leucocyte count 38,000 of which 99% were small mononuclears. In Westphal's case the red cell count was 816,000 and the leucocyte, 24,000. In Bezançon and Labbé's case there were

500,000 red cells per cubic millimeter of which 3250 were nucleated, and 32,000 leucocytes of which 66% were small mononuclears.

The curve of the absolute number of eosinophiles runs in many cases parallel to that of the red blood-cells. During sixteen admissions a definite rise of these cells accompanied some improvement of the condition. The coarsely granular cells, therefore, have considerable value in prognosis since their count is not affected by changes in the volume of the plasma. The highest counts of these cells were found chiefly in those cases which are certainly doing well or after they have already done well. In several cases in which the general condition of the blood changed little the number of eosinophile cells remained fairly constant. In 10 cases these cells fell as the red blood-cells dropped and in 3 they had entirely disappeared at the time of death and had almost in 2 others. These cells may diminish as the patient's condition gets worse even though the red count does not. On the other hand in 8 there was no rise in the count of eosinophiles as the red count rose (plasma changes?) while in 4 there was a rise but without any accompanying improvement. In 1 case, a terminal pneumonia, these cells numbered 220 at death. It may be noted that in the cases with apparent improvement but with no rise of eosinophiles the increase in the count of red cells averaged 43,000 per day; while in cases with definite improvement in the blood condition and with a definite eosinophilia, the average gain of red cells per day was 17,000, *i. e.*, was slow and lasted over a considerable period of time. This would suggest that in the latter cases there is real new formation of blood while in the former the rapid changes in the red count may mean merely plasma changes. The largest counts of eosinophiles were seen in those cases which were gaining very slowly but surely.

A diminution in the absolute number of eosinophile cells may be of ill omen. In 1 of our fatal cases, for example, the red cell-counts had for 15 days before death remained constant (that is, the first of 6 counts was 2,832,000, the last 2,704,000, and the average in all was 2,700,000). The absolute number of eosinophiles, however, at first was 183, shortly afterwards was 180, while toward the end none of these cells were found.

Platelets.—In pernicious anemia the blood-platelets are decreased often to only ⅒ their normal number, while they may be apparently absent. In other cases they are said to be increased (v. Limbeck and Sahli). Grawitz found that they varied. Hayem found the count as low as 25,000 or even 15,000 per c.mm.

The coagulability of the blood in pernicious anemia is usually decreased. The blood obtained by venesection does not separate into clot and serum.

Serum.—The plasma in pernicious anemia loses very little of its albumin. There may be a loss of 50% of the albumin of the whole blood and yet the serum will lose but 8%. Pernicious anemia is quite different, therefore, from the hydremic anemias after hemorrhage and those due to cancer, sepsis (cryptogenetic infections), etc.

The *specific gravity* of the blood averages about 1.030 and may go as low as 1.025.

The *solids* of the blood average but about 9% and the water is increased to even 90%. The loss is in the albuminous bodies and due to the reduction in the count of corpuscles, for the serum in even the severe cases may be practically normal. In the plasma the serum globulin alone is decreased while the serum albumin remains practically normal.

The *blood lipoid* values in anemia were found by Bloor and MacPherson [120] to be "normal, or nearly so, as long as the percentage of blood corpuscles remained above half the normal value. When the percentage was below this level abnormalities appeared which, in the order of their magnitude and also of the frequency of their occurrence were, (1) high fat in the plasma, (2) low cholesterol in the plasma and occasionally in the corpuscles and (3) low lecithin in the plasma.

"The lipoid composition of the corpuscles was found to be normal in all cases. There was therefore nothing in their composition to indicate abnormal susceptibility to hemolysis.

"Removal of the spleen resulted in increased total fatty acids and lecithin in the corpuscles and of cholesterol in the plasma. The results were essentially the same whether the patients had anemia or not.

"The relation between free and bound cholesterol was found to be within normal limits in all cases of pernicious anemia, thus giving little support to the assumption that an abnormally great combination of cholesterol as ester is a factor in the production of anemia.

"The low values of lecithin and the high values for fat which were generally most marked in those cases where the blood corpuscle percentages were lowest are regarded as due to deficient fat assimilation in the blood resulting from the lack of sufficient corpuscles to bring about the change of fat to lecithin which has been found to be one function of the corpuscles.

"While the results offer no certain evidence that abnormalities in the blood lipoids are responsible for anemia, the low values for cholesterol, which is an antihemolytic substance, and the high fat fraction, which may indicate the presence of abnormal amounts of hemolytic lipoids in the blood, are possible causative factors of which further investigation is desirable."

Chlorosis.—Chlorosis is a disease especially of young girls at puberty (hence the desire to relate this disease to some defect of sex hormone formation), the essential blood-feature of which is a reduction in the size and thickness of the red blood-cells. The amount of hemoglobin is, therefore, much reduced, much more than is the count of red cells. These cells show practically no signs of degeneration or destruction. There would seem also to be a polyplasmia. Chlorosis is the best illustration of an anemia due to defective hemogenesis. The absence of hemolysis is shown by the poverty of the urine in pigment and by the absence of jaundice.

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[120] Jour. of Biol. Chem., July, 1917, xxxi, p. 79.

The blood features in chlorosis which deserve special mention are: the remarkably uniform diminution in the size of the red cells; their almost uniform paleness (in the secondary anemias, even of a severe grade, the red cells vary widely in size and in color); the low color-index (lower than in secondary anemias); the lymphocytosis usually present; the infrequent appearance of nucleated reds and the increased coagulability of the blood. And yet chlorosis is more a clinical than a blood picture, since this latter is well simulated by many secondary anemias. On the other hand the clinical picture is so sharp that some speak of chlorosis without blood changes (Laache).

The gross appearance of the drop is very pale, thin, and watery. It clots rapidly.

The count of the red blood-cells is not very much reduced and very low counts are rare, and yet "in over 60% of the cases it is under 4,000,000 cells at the time of the first visit" (Reinert, v. Limbeck). Thayer's average of 63 cases on the first visit is 4,096,000; Cabot's, 4,112,000; Gräber's, 4,482000; while Grawitz's cases varied from 3,400,000 to 4,300,000. The minimum count of Cabot's was 1,932,000; of Thayer's, 1,953,000, and of Hayem's 937,360. Gräber, who claimed that in simple chlorosis there is no diminution in the count and that a diminution would indicate a complication, as ulcer of the stomach, cites one case with 5,700,000 cells. The color of the red blood-cells suggests a marked diminution of the hemoglobin. Their biconcavity is very pronounced, the pessary forms are common and they stain very poorly (Plate I, 23, 24).

The majority of the cells show a quite uniform diminution in size, and yet there are present just enough large, pale, so-called "chlorotic cells" to bring the average diameter up to almost normal (they vary from 5.2 to 11.5μ in diameter, with an average of 7.5μ). Many consider that these large cells are dropsical;—that is, that they are swollen by the water they have imbibed from the plasma. Macrocytes are rare, while microcytes are more common. Schaumann and Willebrand say that at the height of the disease the smaller cells predominate, but during convalescence the large cells. Grawitz, on the other hand, says the cells are largest when the case is at its worst; that these large, chlorotic cells, at the height of the disease, may be very numerous, may even make up one-third of all the red cells.

Poikilocytes and degenerated cells are rare except in the severe cases. The polychromatophilia present is considered by many to mean youth and, therefore, to be a sign of active regeneration (Grawitz). "The granular degeneration does not belong to the picture of chlorosis, but means some complication." Stengel and Pepper, however, think it is common.

Nucleated reds are very rare in chlorosis except in the very severe cases and during the blood crises. They certainly are much rarer than in the secondary anemias. Those present are usually normoblasts, seldom megaloblasts.

It is the marked reduction of the *hemoglobin* which is the characteristic feature of chlorosis. This may be reduced to even 20%. Cabot's average on first visit was 41.2% and Thayer's, 42.3%. The color-index is, therefore, low, averaging 0.5, but in some cases it has been reported as low as 0.3. Secondary anemias never reach this level. The explanation in chlorosis is the small amount of hemoglobin in each cell and the large numbers of small cells. The volume of the red blood-cells is just about half the normal.

The average *leucocyte count* in Thayer's cases was 8467 and in Cabot's, 7485. That is, the count is normal although a leucopenia is not uncommon. This is important in diagnosis since in the secondary anemias a slight leucocytosis is the rule. During convalescence the leucocytes may increase more rapidly than do the red cells and there develops, therefore, even a leucocytosis.

Grawitz and v. Limbeck say that the *blood formula* is normal and that is the present opinion. Those observers who find, even in mild cases, the percentage of small mononuclears about 33% and that of the neutrophile cells correspondingly reduced probably still cling to Ehrlich's first formula, which now is considered incorrect. Some cells resemble myelocytes, but typical ones are very rare. The eosinophile cells are usually somewhat increased, averaging 3.5% and in some cases reach even 9.6%.

Chlorosis is a condition met with much less frequently than a few years ago. During 5 years but 13 cases were admitted to the Johns Hopkins female wards diagnosed as chlorosis. Of these but 2 were at puberty, and the rest were from 17 to 25 years old (relapses?). Of these, the lowest count was 2,600,000, the highest 4,000,000 and the mean, 3,700,000. The hemoglobin varied from 26 to 49%, the color-index from 0.36 to 0.63 (the mean 0.47). The leucocyte counts were 2400 and 3800; between 5000 and 7000 there were 6 cases, while the highest was 8000.

The differential counts made in 7 cases were all practically normal (even that of the case with a count of 2400 was); s.m. 17.2%, l.m. 2.9%, p.m.n. 77.1%, and eos., 1.8%.

It is interesting that when these 9 cases left the hospital all had gained practically the same number of cells, between 900,000 and 1,711,000, the mean, 1,100,000.

The *platelets* are increased in number as a rule; in fact, in no condition are they as numerous as in chlorosis. They also are large in size.

The *specific gravity* of the blood is low, sometimes down to 1.030. This is due to the decrease of hemoglobin. In this disease alone does the specific gravity of the whole blood run exactly parallel to the hemoglobin content. Grawitz states that it varies from 1.035 to 1.045; others say from 1.030 to 1.050. Grawitz says that if it is under 1.035 some complications must be present.

The alkalinity of the blood is normal.

The isotonicity of the cells is low.

In the plasma one finds but few changes. There is no blood destruction and no hydremia. As the case improves the red cell count rises rapidly to normal; that is, "the anemia is first cured" (Gräber), then the light-weight cells are slowly replaced by those more normal in size and weight. Yet all the variations in the count need not necessarily mean a new formation of cells since the first sign of improvement may be an increase of the specific gravity and an increased count due to the dissapearance of some of the plasma, as is indicated also by the early polyuria and by the disappearance of edema. Later, the signs of regeneration appear and also the gradual elimination of faulty cells. Then the leucocytes may rise to even above normal.

Aplastic Anemia.—Aplastic, hypoplastic, or aregeneratory anemia are names applied by Ehrlich to that type of anemia due to decreased blood formation. Clinically it is marked by anemia, a pronounced tendency to hemorrhage and a rapidly fatal course. The blood shows a marked anemia, even 490,000 per cu.mm., while the hemoglobin falls even faster as is shown by the continuous drop in the color-index. None of the evidences of bone-marrow regeneration are seen, such as megaloblasts, normoblasts, anisocytes and stippled or basophilic erythrocytes in the circulation, while the absence of bile pigment in the skin, urine and blood plasma and the absence of hemosiderin in the liver tend to prove that the anemia is due to failure in formation and not to abnormal destruction of red blood cells. The platelets are few or quite absent. There is progressively developing leucopenia nearly always to 2000 but even to 140 white cells per cu.mm. Every type of leucocyte suffers, but the lymphocytes least. The polymorphonuclear cells, including the neutrophiles, eosinophiles and basophiles diminish markedly and progressively.

At necropsy the red marrow is found to be aplastic, showing an increase of fat and a diminution in megaloblasts and normoblasts.[121]

Musser [122] collected 59 cases, including 24 of Cabot.

An aplastic anemia may be due to a toxin which destroys the bone-marrow, as benzol, but would seem a possible and logical termination of any long-standing anemia and represents the bankruptcy of the hematopoietic tissue.

In 1907 we [123] reported a case of probable aplastic anemia which seemed related to the leukemias. The patient, a girl 19 years of age, died after an illness of about 1 month with red corpuscles 724,000, hemoglobin 13% and leucocytes 1920. Six days before death the leucocytes were 3800 and the differential count: s.m. 39%, l.m. and tr. 43%. pmn. 14%, eos. 1%, myelocytes 0.2%, and unidentified cells 2.5%.

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[121] O'Malley and Conrad, Jour. A. M. A., Dec. 6, 1919, vol. 73, p. 1761.

[122] Arch. of Int. Med., 1914, xiv, p. 275.

[123] Johns Hopkins Hosp. Rep., Mch., 1907, xviii, p. 82.

LEUKEMIA

Barker has defined the leukemic state as one in which there is definite proliferation of the leukopoietic tissues, either myeloid or lymphadenoid, and the appearance in the blood of immature white blood-cells, usually in large numbers, the degree of whose immaturity is more pronounced the more acute the cases.

By this definition of leukemia he excludes Hodgkin's disease and the ordinary leukocytoses and lymphocytoses, since in these processes neither the blood picture nor the changes in the tissue characteristic of leukemic states are present. It also excludes the aleukemic lymphadenoses and the aleukemic myeloses (pseudoleukemia) in which, though the histological changes in the hemopoietic tissues may be identical with those of leukemia, the characteristic blood picture is absent. The aleukemic lymphadenoses and the aleukemic myeloses may, however, be closely allied to the leukemic states since acute leukemic states are often preceded by aleukemic stages. Moreover, in the course of an outspoken leukemia the hematological picture may change to that of an aleukemic state.

From the present point of view of the clinical laboratory, leukemia is a disease characterized by the constant presence in considerable numbers in the blood of white cells not found normally in the peripheral circulation. This is certainly true of the mononuclear granular cells and probably also of the non-granular cells present in both forms of leukemia and almost exclusively in one. These cells are supposed to be immature forms of the ordinary leucocytes which because of some disease involving the blood-building tissues are extruded into the peripheral circulation. The essence of leukemia is, therefore, a marked change in the blood formula. There is in leukemia, as a rule, also a great increase in the leucocyte count and yet this is not constant while during the periods in which the total count is normal the diagnosis can often be made from the presence of these abnormal cells alone.

Leukemia has long been rated among the primary anemias although the reasons for this no longer have force. Anemia does, as a rule, develop during the course of a leukemia, not as a necessary part of the clinical picture, but rather of the cachexia which, sooner or later, always develops.

According to the blood picture three forms of leukemia may be recognized.

(1) Lymphatic leukemia, lymphadenoid leukemia, "lymphemia," in which the increase is of the non-granular cells.

(2) Splenomyelogenous leukemia, myeloid leukemia, "myelemia," or "true leukemia," in which there is an absolute increase of all leucocyte

forms and the presence of mononuclear granular cells never normal in the circulation.

(3) Mixed leukemia, in which the features of both of these 2 forms are combined.

Of all 3 forms, acute cases may occur.

Leukemia differs from a leucocytosis, not so much because the white cell-count usually is much higher, for it often is not, but because the blood condition is more permanent and not ephemeral as is a leucocytosis and because of the large numbers of abnormal cells (in leucocytosis there may be a few present).

Splenomyelogenous Leukemia (Plate I).—In splenomyelogenous leukemia all the granular cells are markedly increased, especially the neutrophiles, but also the eosinophiles and basophiles. Abnormal (so far as the circulation is concerned) immature forms of each are present together with all transitions between these and true leucocytes. The non-granular cells are also very much increased, some of which probably are immature forms.

In many cases of this form of leukemia the *total volume* of blood would seem to be increased. This is indicated during life by the dilatation of the veins and later by the autopsy. Towards death, however, a diminution in the total blood volume would seem to occur.

Grossly, the *fresh blood* may look normal even though the leucocytes may almost equal in number the red blood-cells. In other cases it has a pale, rather opaque appearance and flows sluggishly as though it were thick. It is hard to get good smear preparations (which appear granular); the diagnosis has often been made in this way, the fresh blood resembling "chocolate mixed with cream" (methemoglobinemia?). This must be very rare since so many have never seen it. If a larger volume of blood be allowed to settle and coagulate, a grayish-white layer will form on the top of the clot which may suggest the diagnosis. *Coagulation* is slow and in the severe cases sometimes absent.

As a rule the *red cell-count* is diminished (Grawitz said it always is unless some factor is present which concentrates the blood). In none of Taylor's cases was it above 4,000,000. The cachexia, slight jaundice, increased urinary pigment and the deposit of iron in the various organs show the action of some hemolysin, although the anemia may in part be due to the hemorrhages which are so common. Cabot's average count was 3,120,000 and Osler's, 2,285,000. In 9 Johns Hopkins cases with 11 admissions, the lowest count was 1,640,000, the highest 3,800,000, and the mean 2,800,000. It may be almost as low as in pernicious anemia. As the leucocytes increase the reds decrease and vice versa. There are exceptions, however, and the count may remain almost normal for a long time. This oligocythemia may persist during the periods when the leucocyte count is normal and the patient feels better. If the patient were seen then for the first time a diagnosis of pernicious anemia would certainly be logical (Taylor). The subjective condition of the patients certainly depends very little on the mere count of the red cells for they feel well enough to go home when this has changed very little from that on admission.

The anemia is of the chlorotic variety, *i. e.*, the cells are light in weight. They show remarkably little degeneration, although endoglobular areas do appear. In some severe cases the reds are quite normal. Microcytes and macrocytes are rare; a few poikilocytes are found in all cases. The polychromatophilic degeneration and the basophilic granules are common, yet are never marked. Biermer's test was found positive in 2 cases.

There is no disease in which normoblasts may be found more constantly or in greater numbers than in myelogenous leukemia, even in the cases with a mild anemia, yet their absence is not against this diagnosis. Megaloblasts, and even gigantoblasts 20μ in diameter, are sometimes found. This megaloblastic feature of the blood while common in leukemia is not as marked as in pernicious anemia. Microblasts also are met with. Karyokinetic figures in all stages of division may be best studied here. Of Taylor's 16 cases, in 2 the number of nucleated reds varied from 60,000 to 70,000 per c.mm. and 1 of the first effects of the arsenic was to reduce their number. It is of interest that marked rises in the white count are accompanied by rises in the number of nucleated red cells also.

The *hemoglobin* is reduced, the color-index being about 0.6. Osler's average of hemoglobin was 42%. In 9 recent cases the mean Hb was 30% and the mean index, 0.54. The hemoglobin is hard to estimate since the leucocytes render the blood quite opaque.

From the appearance of the leucocytes of the fresh specimen of blood the diagnosis of myelogenous leukemia may sometimes be made at a glance, not so much because of the large number of leucocytes as because of the large number of immature cells which normally are never present. The count in a simple leucocytosis may be as high as that in some cases of leukemia while in some cases of leukemia the count is normal. It is the blood formula which is important; it is the large and constant number of abnormal cells. In some post-febrile conditions the blood formula may for a short time suggest leukemia since it contains a few neutrophile myelocytes but rarely if ever does it contain eosinophile myelocytes or an increased number of basophiles.

Counts of 500,000 leucocytes per 1 c.mm. are not rare. Cabot's average on the first visit was 438,000 and Osler's, 298,700. While the counts in any given case may vary, they do not as much as might be supposed. The daily counts maintain approximately the same level for weeks. We have not seen as much daily variation as some have mentioned. We counted the blood of several at short intervals. In one case counted each 4 hours the counts were 146,000, 134,000, 140,900, and 143,200. More marked variations do occur, as in 1 case with 122,500 at 10 A.M. and 235,000 at 4 P.M. of the same day. Some cases have quite high counts, over 400,000; but of the most the counts range from 100,000 to 300,000 (63% of 51 cases), while in fewer are they below 100,000. The same case on different admissions may have very different counts, but during any one stay in the hospital it keeps within narrow limits. There are periods when the count is normal, yet even then the differential count will usually give the diagnosis. (In 3 of Taylor's cases, however, there were no qualitative changes.)

All of the cells of normal marrow appear in the blood in myelogenous leukemia. Among these the neutrophile myelocytes predominate. Some of these myelocytes are very large, even 30μ in diameter (Cornil's myelocytes), with a large chromatin-poor nucleus, often in an eccentric position, which stains so palely that it is hard to make out. These cells are seen only in leukemia and in some diseases of children. Other myelocytes are about the size of an ordinary leucocyte and have a round nucleus which stains well. This is the form seen in the inflammatory leucocytoses. And, finally, one usually finds some dwarf myelocytes about the size of a red blood-cell. Mitoses of the nuclei of myelocytes are more or less common. The number of granules in myelocytes varies considerably; the protoplasm of some is full, others have but a few, while there are some concerning which there is doubt whether they are granular or not. Grawitz called attention to large non-granular cells with a large pale nucleus (which also may appear free of protoplasm) which disintegrates rapidly; others are of medium size with basophilic protoplasm which stains intensely and a medium-sized nucleus; in other similar cells beginning granulation can be seen.

Eosinophile myelocytes are present sometimes in large numbers but they are never as numerous as is the finely granular form. All transitional forms are seen between these and eosinophile leucocytes.

While the *polymorphonuclear neutrophiles* are relatively diminished (Cabot's average was 46%) their absolute number may reach even 50,000. Anomalous forms are common: some are very large, even 20μ in diameter, while some are small, or dwarfs, 4μ in diameter. This variation in size never appears in a leucocytosis. Again, cells with unusually shaped nuclei are found and cells with more than 1 form of granule. In 1 case all the cells were described as non-granular. The plasma is full of free granules from many cells which have disintegrated.

The percentage of *lymphocytes* is reduced, the average being 10.6%, but their absolute number usually is increased. These cells vary much in size. Among them are some which can with difficulty be told from myelocytes. One finds also the large mononuclear cells which are numerous enough in the marrow but which never reach the blood normally or in other diseases than leukemia. Some have very irregular shapes, some a few granules.

The *large lymphocytes* have a scanty, ragged protoplasm and a large, chromatin-poor nucleus. These are Fränkel's unripe cells, supposed by some to be characteristic of acute leukemia but which appear also in the chronic types. Large mononuclears, both those of the normal blood and those resembling myelocytes, appear in large numbers. Of the latter the nucleus is often very basophilic. Their protoplasm is finely fibrillar and distinctly basophilic or acidophilic. These cells before Ehrlich's stain was used were reckoned as myelocytes.

Large phagocytes (splenic cells?) are sometimes present and in one case numbered 1.2% of the 216,000 leucocytes.

In this disease there is usually an absolute increase of the *coarsely granular cells.* Ehrlich indeed stated that he would not make the diagnosis of leukemia unless more than 250 of these cells per 1 c.mm. were present. Since then several cases of leukemia have been reported[124] in whose blood at times not a single eosinophile cell could be found and others in which these cells fluctuated much in their numbers. As a rule the minimal number of eosinophiles in leukemia is about 3000, the average percentage 5.1 and the average absolute number 11,000. All forms of these cells corresponding to the finely granular cells occur: The large eosinophile myelocytes (formerly said to be the characteristic cell of leukemia), the medium-sized and the dwarf eosinophile myelocytes and the ordinary leucocytes. The eosinophile myelocytes may in this disease make up the majority of the coarsely granular cells.

Ehrlich considered that leukemia is the one condition in which there is an absolute increase of the number of *Mastzellen.* They may even outnumber the eosinophiles. In one of Lazarus' cases they reached 47%; in one of Cabot's 10%, while Taylor mentions a case with an absolute count of basophiles of 140,000. Taylor also states that in two cases no Mastzellen were found.

Charcot-Leyden crystals may be found in leukemic blood which has stood for a while, but they may be found also in fresh blood obtained by splenic puncture. Some observers, however, have never found them. They are normal in the bone-marrow and are present wherever the eosinophile cells are increased.

Bezançon and Labbé say that *leucin spherules* also will separate spontaneously from leukemic blood.

Ehrlich considered that the *diagnosis of myelogenous leukemia* could be made from the examination of a smear alone provided one found neutrophile myelocytes, eosinophile myelocytes, an absolute increase of eosinophiles and of Mastzellen, atypical cells, especially the dwarf eosinophiles and neutrophiles, cells in mitosis and, lastly, a large number of nucleated reds. Yet, for a while at least, any one of these points may fail.

Many of the leucocytes in leukemic blood show signs of degeneration. Ewing considers that eosinophile myelocytes with granules of very unequal size and density of stain are pathognomonic of myelocythemia. The protoplasm of some cells is swollen, hyaline, or vacuolated. One finds many nuclei surrounded by free granules, the protoplasm evidently having disintegrated. Karyolysis, vacuolation and karyorrhexis of the nuclei are common; pycnosis perhaps less so.

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[124] See Simon, Am. Jour. Med. Sci., No. 125, 1903.

The diagnosis of leukemia cannot be made from the total white cell-count alone, since the count in pneumonia may rise above 100,000 and that in leukemia may drop to normal; and yet the former high counts are very temporary, while those of leukemia continue for a long time. The mere presence of myelocytes is not sufficient for diagnosis, since in cases of extreme leucocytosis a few true myelocytes may be found. These, however, are very few in number, are about the size of the ordinary leucocyte and are never the very large cells which are seen in leukemia; also in leucocytosis the eosinophiles and Mastzellen are not increased. In children especially the diagnosis is difficult. Indeed autopsy alone may decide it.

The white cell-count of 1 case on first admission was 443,000. Fourteen months later the patient was readmitted with a count of 9700 and discharged 20 days later with one of 100,000. The lowest count during this admission was 6000, of which 3.8% were small mononuclears, 3.6% large mononuclears and transitionals, 70.8% polymorphonuclear neutrophiles, 3.8% eosinophiles, 8% neutrophile myelocytes and 7.6% Mastzellen. There were 2.3 normoblasts, 15 intermediates, and 5 megaloblasts per 1000 leucocytes. Hence even on that day a diagnosis could have been made from the blood formula alone.

With improvement in the condition the count may drop to normal. At such times the formula may remain leukemic or may become normal, in which case the condition would closely resemble pernicious anemia. And indeed cases have been reported as changing to pernicious anemia and *vice versa*. Following the long-continued use of arsenic and of benzol the count may drop in a remarkable way usually to rise again after the drug is discontinued. Turk[125] mentions a case in which after arsenic treatment the leucocytes, which had ranged from 258,000 to 370,000, dropped to from 3000 to 6000 of which 0.5% were myelocytes and 6.6% Mastzellen. It is a question how much improvement these low counts indicate since they may be due to "exhaustion" of the bone-marrow. Following X-ray treatment remarkable drops have been reported, *e. g.*, from 693,000 to 6300. (In this case the leukemic character of the blood was never lost.) But the most remarkable falls have followed the use of benzol. In the case reported by Barry and Ketcham these cells fell from 150,000 to 3000.*

Radium also has a most definite effect on the blood picture.[126] In cases which have received no previous similar treatment the number of white cells usually begins to fall in from 24 to 72 hours after the radium is applied. The decrease in the leucocytosis is often rapid and continues for days and even several weeks after the radium was administered. In one patient the white count dropped from approximately 100,000 to 6100 in 25 days, radium having been given on the first and thirteenth days only. There is a change also in the differential count. Myelocytes and immature forms of polymorphonuclear leucocytes become less prominent and a larger proportion of adult polymorphonuclear cells is found. Patients with an anemia, who respond well to treatment, show a rise in hemoglobin and in the red cell-count. On the other hand the development in a case under observation of an anemia and the occurrence of many nucleated red cells is to be regarded as a serious sign. An important point to bear in mind is that the development of anemia may be the result of too much radiation. The infectious diseases, especially typhoid fever, influenza, miliary tuberculosis, *et al.*, have a remarkable effect not only on the blood picture but also upon the blood-forming organs of leukemic patients. In Dock's case[127] of grippe, the cells fell from 367,000 to 5000 but in 6 weeks had returned to 157,000 and in 1 year were 461,000. The fall is sometimes extreme as from 40,000 to 470. Some of these cases preserve in the low counts the leukemic formula, others do not. When the count falls there is also temporary reduction in the size of the blood-building organs, but not always, as in a case reported by McCrae. Cases are on record of leukemic patients who have died of infectious diseases and in which at autopsy all signs of leukemia had disappeared from the bone-marrow. In other cases, however, the count rises instead of falls. In Müller's case of sepsis the leucocytes dropped from 246,000 to 57,300 and then rose; in v. Limbeck's case of pneumonia they fell from 140,000 to 43,500 and then when the other lung became involved, rose to 172,000. As the count drops the percentage of polymorphonuclears rises and the picture thus approaches that of a leucocytosis.

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[125] Deut. med. Wochenschr., 1904, No. 50.

[126] Peabody, Boston Med. and Surg. Jour., Dec., 1917.

* Jour. of Ind. State Med. Assoc. Dec., 1916.

Late in the disease there may be a marked predominance of large non-granular leucocytes. It is possible that some of these are myelocytes without granulation, the body having lost its power to form the neutrophile material (Ehrlich).

In myelogenous leukemia the count of *platelets* is markedly increased.

The *water content* of the blood is increased to from 81 to 88%.

The *specific gravity* of the blood is low, even 1.036; that of the plasma is about normal.

The *alkalinity* of the blood in leukemia is somewhat decreased by the organic acids formed from the breaking down of leucocytes. Formic, acetic, lactic and succinic acids have been found in the plasma. The *xanthin bodies* of the plasma are increased. *Deuteroalbumoses* have been found. These are not present in lymphatic leukemia and are supposed to be digestive products of the leucocytes by a ferment provided by the polymorphonuclear cells. Taylor says that the nitrogen of the leucocytes is almost double.

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[127] Am. Jour. Med. Sci., 1904, vol. cxxvii. This is a very exhaustive study of this subject with a review of 50 cases.

Nucleo-albumin is found in the serum, and 22.6 mgms. of *uric acid* per 100 c.c. of blood. The coagulability of the blood is sometimes so increased that the red blood-cells cannot be counted with a pipette.

Lymphatic Leukemia (Lymphemia). (Plate II, A, B, C).—In lymphatic leukemia there is a marked increase of the mononuclear non-granular cells. Despite the name, these cells are not all typical "lymphocytes" in morphology, perhaps not in origin, but are mononuclear, non-granular cells of many sizes and forms.

While a variety of these cells may be present 1 particular type usually predominates in each case. In most cases they all are small with a very narrow ragged rim of protoplasm; in others they all are of the large lymphatic type; in other cases the majority resemble the transparents and in still others the transitionals of Uskow. In some cases the protoplasm of these large cells seems more basophilic; in others more acidophilic. Sometimes enormous cells are found.

In lymphatic leukemia there always is a proliferation of lymphatic tissue somewhere in the body. In chronic cases the peripheral lymph-glands are enlarged yet in more acute cases none of these glands may be palpable. In some cases there are large masses of lymphatic tissue along the intestines while in still others the lesion would seem to be limited to the bone-marrow. Some interesting cases begin with large peripheral lymph-glands and a normal blood picture but later, as the leukemic condition appears, these glands diminish in size. One patient in September had normal blood and a general glandular enlargement but the following January he was admitted with smaller lymph glands and a leucocyte count of 110,000, chiefly small cells.

Pappenheim suggested that the leukemia may not begin until the disease of the lymphatic tissue has reached the marrow.

The *anemia* is more often marked in lymphatic than in splenomyelogenous leukemia and yet the red cell-count may remain normal for some time. Later, however, a cachexia is almost inevitable and with it an anemia. In two chronic cases the count persisted above 4,000,000 during a long stay in the hospital and until death. On the other hand the anemia may be extreme as in a remarkable case reported (verbally) by Dr. Hazen, of 18 months' duration, with red cells 960,000 and leucocytes 250,000, nearly all of them small lymphocytes. Cabot's average on the first admission was 2,730,000; Osler's 2,294,000; that given by Hirz and Labbé is 1,829,000 and that by Petit and Weil, 1,292,000. The red cell count is said to be lowest in those cases which autopsy shows have most involvement of the bone-marrow and also in the more acute cases and in these there always is little peripheral glandular enlargement. *Nucleated reds* are rarely found. Von Limbeck describes them as astonishingly scarce. In some very severe cases, however, they may be as numerous as in splenomyelogenous leukemia. In 1 of our cases in which the total white count was 12,000 there were 150 normoblasts, 169 intermediates and 20 megaloblasts per 1000 leucocytes, *i. e.*, a typical megaloblasts crisis.

The red cell-counts may remain very constant, while those of the leucocytes are fluctuating widely. In 1 case on 1 day there were 2,640,000 red cells and 105,000 leucocytes, 9 days later these counts were 2,750,000 and 328,800, 2 weeks later 2,892,000 and 410,000, finally in 2 days 2,928,000 and 480,000.

In a case with pleural and ascitic effusions (chylous) repeatedly tapped and with profuse diarrhea, who died of streptococcus septicemia, the white cells showed very slight variations. On admission they numbered 133,400; then rose to 242,000 and at death numbered 133,000; the red cells of this case numbered 4,912,000 on admission and 5,340,000 at death. Several counts were above 5,000,000.

The *hemoglobin* is diminished (Osler's average was 37%).

The *leucocyte counts* averaged 144,800 (Osler) and 141,000 (Cabot). It may be as high as in the myelogenous form, but this is rare. In this form also there may be aleukemic periods which may last even 6 months. Just before death the count usually rises. One of our cases had a leucopenia of even 1900 cells during the 10 weeks before death.

The mononuclear cells are usually over 90%, sometimes 98%, and in 1 of Osler's cases 99%, of the total white-count. A marked feature of the blood picture are the degenerated leucocytes. Even 10% and just before death even 75% of them may show degenerations of the protoplasm, or pycnosis and fragmentation of the nuclei. It is noteworthy that so few of these cells in the blood show mitotic figures since in the bone-marrow their proliferation is very active. Grawitz classifies the cases as: those in which the small mononuclears, predominate (many of these are very small, even smaller than red blood-cells, their protoplasm intensely basophilic and scanty, the margin ragged and degenerated, their nuclei round or indented and even fragmented with sharp margins and often containing clear areas); those with a predominance of medium-sized cells with basophilic homogenous protoplasm; and those in which the cells which predominate are very large and are, for the most part, degenerated. Yet all these forms may occur together in the same case, and vary in their relative percentage at different times. Roser thinks that in those cases in which the lymph-glands are particularly involved it is the smaller cells which are increased (*e. g.*, 99% of 117,000), and that in those in which the lesion is particularly of the bone-marrow, the larger cells. Grawitz mentions a case in which the percentage of the larger cells increased simultaneously with a decrease in the size of the lymph-glands. Wolff thinks we should separate lymphatic from lymphoid leukemia, the former being of lymph-gland origin, the latter myelogenous.

Polymorphonuclear granulated cells are rare in the circulating blood. Eosinophile cells are usually absent. In a pure case no myelocytes are present, although it would be hardly wise to call the case mixed leukemia if one were found. Mastzellen are as a rule absent. In this form of leukemia an acute infection may cause a drop of the total count or a true leucocytosis. On autopsy on cases of lymphatic leukemia who died of acute infection no leukemic lesions have been found.

During the periods when the count is low the mononuclear cells may be even 90% of the total count. In Wende's case[128] complicated by a streptococcus infection the white cells dropped from 45,000 to 1600, but the percentage of small mononuclears only from 95.3 to 88%. As the result of an acute infection a few myelocytes may appear. In other cases, however, an infection causes a marked increase in the count. In Müller's cases, complicated by chronic septicemia, *e. g.*, it rose from 180,000 to 400,000.

A man was admitted to this clinic with double tertian malaria and a lymphatic leukemia of 105,000 cells (small monos. 83.6%; large monos. and tr. 7%; pm. n. 5.8%; eosinoph. 0.2%). One week after the malaria was cured the count rose to 328,000 and 2 weeks later to 480,000 with 97.2% small mononuclears. At this time there were 3 normoblasts, 3 intermediates and 4 megaloblasts per 1000 leucocytes.

Von Limbeck considers that the blood picture alone is not enough for diagnosis of lymphatic leukemia since in some cases of sarcoma the blood presents a similar picture.

There is a good reason for separating the acute leukemic states from the chronic leukemic states, for the mode of onset, the clinical symptoms, the course, the duration, the blood picture and at autopsy the histological changes, differ. Whether the etiological agent is the same for the 2 groups of cases is not certain (Barker). It is possible that they are identical though many investigators believe that the etiological agents are entirely different from one another. In favor of the identity of etiology is the fact that a chronic leukemia may have an acute onset or an acute termination.

Acute Leukemia.—Acute leukemia is a form of leukemia characterized by its brief course,—from 6 days to 9 weeks (leukemia acuta et acutissima),—the severity of the symptoms, frequency of the hemorrhagic diathesis, the rapidly developing cachexia and the rapid death. It occurs chiefly in young persons. The great majority of the cases are of the lymphatic type, "acute lymphadenoid leukemia," but a few of the myelogenous variety, "acute myeloid leukemia," have recently been reported while other cases are best described as mixed.

Cases of the *acute myelogenous forms* are collected by Gardinier[129] who reports 1 and reviews 11 others.[130] In all of these cases the anemia is extreme, the red blood-count even below 1,000,000 cells. In Arneth's case the red cell-count was 256,000 per c.mm. and the hemoglobin 10%.

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[128] Johns Hopkins Hosp. Bull., October, 1904

[129] See also Billings and Capp, Am. Jour. Med. Sci., 1903.

[130] Am. Jour. Med. Sci., vol. cxxii, 1901.

The cases of *acute lymphatic leukemia* are well reviewed by Rosenberger.[131]

Acute leukemia in children is reviewed by Churchill,[132] who reports 1 case and reviews 28 others. The disease occurs even in the new-born child. The lowest red count was 750,000 (after a severe hemorrhage). The leucocytes varied from 6000 to 810,000 (in a 20-month-old child). The counts were always lowest just before death, which a falling count portends. Of these 29 acute cases in children, 25 were lymphatic (2 of the small-celled type, 3 large, 1 mixed), 1 was myelogenous, 2 were mixed and 1 was uncertain. In Churchill's case 99% of the white cells were small mononuclears many of which were degenerated. The anemia is profound. It is of interest that the more acute the case the less evidence is there of involvement of lymph-glands and spleen.

A good illustration of this type is Pfannkuch's case, which ended fatally in 3 days. The reds numbered 2,500,000 and the leucocytes 1,000,000 (s.m. 76.5%; neutrophilic myelocytes, 10.6%; neutrophile leucocytes, 12.2%). In Türk's case, a good illustration of the myelogenous form, the red cell count was 1,060,000; hemoglobin 19% and the leucocytes 42,000 (s.m. 14%; pmn. n. 32% and myelocytes 47%).

The blood picture in a variety of conditions may closely resemble acute myelogenous leukemia. These are: an acute exacerbation of a chronic myelogenous leukemia; a lymphatic leukemia complicated by an acute infection; an acute lymphatic leukemia of the large-celled variety (since it is not easy to distinguish these cells from myelocytes); an acute infection causing grave anemia, in which case even 14% of the leucocytes may be myelocytes; an acute exacerbation of pernicious anemia; and, finally, malignant disease of bone-marrow. A very large number of nucleated reds would suggest this last condition (Billings and Capps).

In many cases the clinical picture of acute leukemia is that of an acute infection and very likely in the future such cases will not be grouped under the leukemias.

There is no type of cell characteristic of acute leukemia: Frankel's unripe cells are common, but are found by no means exclusively here. In 3 of McCrae's 5 cases the small lymphocytes predominated. The cells in any given acute case are likely to be much more uniform than in a chronic case, yet this is by no means always true. (Plate II, C.)

The red cells show few changes. As a rule nucleated reds are scarce, yet in Herrick's case they numbered 1,800 per cubic millimeter, of which some were megaloblasts (but see McCrae's case). The falling count, showing rapid blood destruction, may be a striking feature.

In other cases the leucocyte count is not even above normal although the percentage of lymphocytes is quite high (Klein). Such cases may at first resemble pernicious anemia.

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[131] Am. Jour. Med. Sci., 1904, vol. cxxviii, p. 583.

[132] Ibid., 1904, vol. cxxviii.

The 5 cases of acute lymphatic leukemia in the Johns Hopkins clinic have been reported by McCrae.[133] Their duration, which varied from 12 days to 8 weeks, averaged 6 weeks. On admission the hemoglobin averaged 35.4%; the reds, 1,822,000 and the leucocytes, 104,000. The highest white count was 326,000 (hemoglobin 45%; reds 3,000,000); the lowest 57,800 (reds 748,000; hemoglobin 16%). In 1 case in 15 days the reds fell from 3,000,000 to 1,450,000. The color-index is high, from 0.93 to 1.4. The small mononuclears varied from 94.2 to 99.4% and in 3 the small lymphocytes were the prevailing cell, unusual in this form of leukemia. In 1 case actively motile, large lymphocytes were seen. Nucleated reds were absent in 2 cases, were present (2 per 1000 leucocytes) in 3 and in the fifth case there were 310 per 1000 leucocytes (*i.e.*, 3720 per c.mm. of which 7% were megaloblasts and 48% intermediates). McCrae emphasizes the high color-index of these and of the cases in literature and finds that of 45 cases in 24 the red count was below 1,500,000 and in 38 of the 45 it was below 2,500,000. Of 40 cases from literature, in 20 the color-index was 1 or above 1. The low red count and the high index of the primary anemias seem to him a special feature of this type of leukemia. A most remarkable case was admitted with 752,000 red cells, hemoglobin 17% and 880,000 leucocytes of the large-cell type.

In 13 cases of acute leukemia collected by McCrae,[134] the anemia was severe (the highest red count was 2,350,000; the lowest, 1,000,000) and the color-index high. The red cells were of normal appearance. No nucleated reds were found in 7 cases, a few in 4 and megaloblasts in but 1. The leucocytes varied from 21,000 to 209,000. The absolute number of polymorphonuclears was about normal in all cases. In these cases the anemia was the important feature. The leukemia would not have been suspected without blood examination.

The cause of leukemia is still to be discovered. Auer [135] described very interesting rods in the cytoplasm of the large mononuclear leucocytes of a case of acute leukemia. Mention may be made of Löwit's organism (Fig. 134). In cases of splenomyelogenous leukemia he described bodies in the large and small mononuclears, both non-granular and granular, rarely in the polymorphonuclear cells, never in the red cells and sometimes free in the plasma, which he named Hemameba leukemiæ magna. Their size varies much. Some of the groups of such bodies he considers evidence of multiplication. They can be stained by a particular method. Löwit found in the blood-building organs navicular and crescent-shaped bodies which suggested the coccidia and the hemosporidia and which first suggested to him the possible parasitic nature of the disease. He did not find them in other conditions, and denied that they are artifacts. Finally he states that he got positive results from rabbit inoculation.

In 1 of 5 cases of lymphatic leukemia he found Hemameba leukemiæ parva, which was smaller than the preceding and apparently more ameboid. These he found especially in the blood-building organs, rarely in the peripheral blood.

Türk considered these parasites of Löwit to be much altered basophile granules or artifacts. By unanimous consent the question has been allowed to drop and yet if all these bodies which we have seen in the blood specimens stained with his method are artifacts they certainly were beautiful pseudoparasites.

The frequent association of acute lymphatic leukemia with *tumors of the thymus* has been emphasized by Major[136] and Asbury.*

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[133] Brit. Med. Jour., February 25, 1905.
[134] Johns Hopkins Hosp. Bull., May, 1900.
[135] Am. J. Med. Sci., June, 1906.
[136] Johns Hopkins Hosp. Bull., Sept., 1918, xxix, 331.
* Jour. of Ind. State Med. Assoc. Dec. 1920.

Mixed Leukemia.—The cases of mixed leukemia may best be described as a lymphatic leukemia with a considerable number of myelocytes, both eosinophile and neutrophile, also present. Since a few myelocytes may occur in lymphatic leukemia the term should be used with caution.

Pseudoleukemia.—Under the heading pseudoleukemia have been grouped a great variety of diseases which suggest leukemia clinically but not hematologically. Of first importance under this heading are the early stages of lymphatic leukemia, before the blood changes appear, and cases of leukemia during the aleukemic periods. But the group includes Hodgkin's disease (which we believe can be excluded by removing a superficial gland), tuberculosis of the lymph-glands, lympho-sarcoma and malignant lymphomata (which the pathologists say can be recognized anatomically). Others include splenic anemia. It is, therefore, the opinion of some that the existence of a "true" pseudoleukemia is still to be proved (Reed).

HODGKIN'S DISEASE.—The blood features in Hodgkin's disease are those of cachexia. At the onset the count may be practically normal and remain so for months despite the rapid growth of the lymph-glands. Then slowly develops an anemia of secondary type, often extreme, with at the end a count as low as 1,522,000 with degeneration of the red cells, nucleated reds and poikilocytes (which are noticeably rare except at the late stages). There is a slight leucocytosis averaging about 12,000. In the 8 cases reported by Reed the red count on admission varied from 3,232,000 to 5,264,000. In 1 case it was 2,670,000 but afterwards improved. These cases were, therefore, somewhat anemic, while 2 showed a severe anemia of the secondary type.

In 2 of the 8 cases the small mononuclears were absolutely increased but the lymphocytosis of Pinkus is by no means constant. In 1 case the count of small mononuclears was 5304 per c.mm. (38.6%) and the next highest was 4600 (36.8%). In 2 cases the small mononuclear count was low; in one 310 (2%) and in another 940 (9.4%). Grawitz considers that the differential count is of no aid in the diagnosis of Hodgkin's disease but is in the prognosis since a slight increase indicates improvement and a decrease, the reverse.

TUBERCULOSIS OF THE LYMPH-GLANDS.—In cases of general glandular tuberculosis there may, for a time, be a normal red and leucocyte count but one more often finds a secondary anemia accompanying the cachexia. Some of the lowest white counts of all have been reported in this condition, *e. g.*, 300 leucocytes per cubic millimeter (Futcher).

We studied 12 cases of this disease in the Johns Hopkins clinic. Four had a leucocytosis of from 11,000 to 29,000 and a slight secondary anemia. In 2 cases the red cell counts were 3,600,000 and 3,700,000; and in 6 cases it varied from 4,000,000 to 5,000,000; the hemoglobin was so much reduced that in 6 cases the index was below 0.6. In this disease a leucocytosis as a rule means a secondary infection.

A case like the following is a puzzle for diagnosis. The woman, aged 50 years, was first admitted with a red count of 4,000,000, hemoglobin 50% and leucocytes 8000. She had tuberculosis of the lungs and swollen lymph-glands, 2 of which had been removed with an interval of 1 year between operations and both pronounced tuberculous. She had night-sweats and lost weight. Three months after the above blood-count the glands began to swell enormously. The red cells then were 3,000,000 and the leucocytes 80,000, 96% of which were polymorphonuclear neutrophiles. A little later the count rose to 120,000. The lymph-glands and spleen became enormous. She received X-ray treatment and in 3 weeks the leucocytes fell to 16,000 and the reds to 2,100,000. She died soon after.

Leukanemia is the name given by v. Leube to a group of cases which have the features of both acute leukemia and of pernicious anemia. In the blood of cases with a severe anemia appear nucleated reds of all varieties and a normal or increased white count. In some of the cases many myelocytes appear present but no eosinophiles while other cases resemble lymphatic leukemia. The anemia usually precedes the increase in the count of the white cells. This blood picture is met with in a variety of conditions [137] including injuries, hemorrhages, intoxications, infections, malaria, malignant growths, etc.

BLOOD IN ACUTE DISEASES

Malaria.—The presence of an anemia is important in the diagnosis of malaria since it is one of the earliest symptoms. In an acute case the count of red cells may, within a few days, drop from 5,000,000 to even 500,000. This is the result both of the direct destruction of the corpuscles by the intracellular parasites and of the action of a toxin which can produce rapid hemolysis. Such cases are, however, very rare. In the average case of tertian and quartan malaria the red cell count decreases slightly after each paroxysm but in the æstivo-autumnal fever with chills there may be a drop of 1,000,000 cells after a single paroxysm and a further fall between the chills.

In 54 cases of æstivo-autumnal malaria the red cells numbered between 1,000,000 and 2,000,000 in 2 cases; between 2,000,000 and 3,000,000 in 12; between 3,000,000 and 4,000,000 in 20; between 4,000,000 and 5,000,000 in 12; and above 5,000,000 in 8. In 56 cases of tertian malaria the figures for these same limits were 1, 10, 28, 13, and 4 respectively. The mean count for each was 3,500,000. It is seen that in the Baltimore cases at least these 2 forms differ but little. In this climate the pernicious cases are rare.

In Grass's case there was a loss of 4,000,000 cells in 6 days. In one case the count at the end of 30 days was 5,000,000. The greatest fall in the count follows the earliest paroxysms, less later, until finally the count remains almost stationary despite repeated paroxysms. In cases with pernicious malaria and hemoglobinemia the anemia becomes grave and poikilocytes, endoglobular degenerations, occasional shadows, fairly numerous nucleated reds, increased platelets and a leucocytosis appear.

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[137] Luce, Deut. Archiv. f. klin. Med., 1900, vol. lxxvii, p. 215.

The regeneration of the cells in tertian and quartan malaria is rapid and anemia develops only in long-standing cases. In æstivo-autumnal malaria the recovery is slower, the new cells are pale and abnormal in size and shape, nucleated reds are common, regeneration is slow and a grave anemia may result. This slowness in regeneration is due in part to the extensive necrosis and resulting fibrosis of the bone-marrow, which may be the chief seat of the infection, and to the accumulation of pigment in this tissue.

The leucocyte count in malaria is almost always below the normal except in the grave pernicious forms. It rises slightly (to 6700, some say to a true leucocytosis) just before the paroxysm, and then falls steadily even to 2000 or even 1000 cells (average 2300) reaching a minimum at the time the temperature is subnormal.

In 82 recent cases of æstivo-autumnal malaria the leucocyte counts were: from 1000 to 2000, 3; 2000 to 3000, 8; 3000 to 4000, 21; 4000 to 5000, 15; 5000 to 6000, 14; 6000 to 7000, 8; 7000 to 8000, 4; 8000 to 9000, 2; 9000 to 10,000, 2; above 10,000, 5. (In 1 of these, a pernicious case, the count was 14,500. The mean count was 3500.)

In 70 cases of tertian malaria the figures for these same limits were 2, 5, 11, 18, 10, 10, 5, 2 and 2; above 10,000, 5. The highest count was 16,500 and the mean 4500.

The differential count in cases of malaria shows a relative decrease of the polymorphonuclear neutrophiles and an absolute increase of the endothelial leucocytes. The mean averages found by Thayer are: s.m. 16.9%; l.m. 16.9%; pmn.n. 65%; eos. 0.9% and in grave cases 2 to 3% of myelocytes (Cabot). The increase of the large mononuclears (endothelial cells) is very pronounced in the apyretic periods and usually absent in the pyretic periods. If in a case of intermittent fever these cells do not increase as the temperature falls the evidence is against malaria. The high percentage of these cells is very valuable in the diagnosis of cases which have been taking quinine and therefore have no parasites in the peripheral blood. Stevens and Christophers say that in the Tropics a blood formula with 15% or more of large mononuclears indicates malaria, while with 20% or more one can almost always find the parasite. This group of endothelial cells, which vary in size from lymphocytes to the largest cells of the blood, are slightly ameboid and distinctly phagocytic, in fact are the chief phagocytes in malaria. Many of them contain pigment granules. Such cells are almost as valuable in diagnosis as is the parasite itself.

Pigmented endothelial leucocytes may be found in the peripheral circulation in cases of æstivo-autumnal malaria most of the time but in tertian and quartan malaria only immediately after a paroxysm. They are said to become necrotic rapidly and so to disappear soon from the circulation.

Stevens and Christophers cite Bastianelli's fatal comatose case of æstivo-autumnal malaria with s.m. 19.1%, l.m. 41%, pmn.n. 39%, and eos. 0.06%; also Panse's case with a temperature of 37.2°C., and s.m. 18.1%, l.m. 26.4%, pmn.n. 55.3%; and another case with temperature normal, with s.m. 14.8%, l.m. 46.7%, and pmn.n. 38.5%.

In 1 of the Johns Hopkins hospital cases of tertian malaria the leucocyte count was 16,500 of which 38.3% were large mononuclears. In a case of æstivo-autumnal malaria the leucocyte count was 6000 of which 26% were large mononuclears. In another case with 4000 white cells, 22% were endothelial cells.

A leucocytosis is rarely met with in malaria, except in the pernicious forms. In one case in that clinic the count one hour before death was 50,000 of which the large mononuclears and transitionals were 18%, and the polymorphonuclear neutrophiles, 58%. A polymorphonuclear neutrophile leucocytosis may develop with an attack of malarial hemoglobinemia and persist for some time. Those seen during the death agony are due to complications. A definite leucocytosis, sometimes with increased eosinophiles and with myelocytes, is common with the post-malarial anemias.

Bignami and Dionisi classified the anemias of malaria as follows: (1) The secondary anemia of the chlorotic type which follows acute malarial fever in which there are a few nucleated reds, leucopenia and an increase of the large mononuclear leucocytes; (2) cases resembling primary pernicious anemia, usually fatal, with extreme oligocythemia, marked poikilocytosis, high color-index, nucleated reds usually megaloblasts, leucopenia and lymphocytes relatively increased; (3) rapidly fatal cases without any signs of regeneration which may have started like a simple secondary anemia. This anemia is very similar to that which follows a severe hemorrhage. (4) Chronic, grave, secondary anemias of the chlorotic type, without nucleated reds and with leucocytes much reduced. This is seen in chronic malarial cachexia, and is due in part to degenerative changes with sclerosis and pigmentation of the bone-marrow. (Thayer).

Septicemia.—That septicemia does not always cause a leucocytosis is seen in typhoid fever and acute miliary tuberculosis, but those due to pyogenic organisms usually do. In the streptococcus and staphylococcus septicemias, *e. g.*, the puerperal infections, the anemia develops more rapidly than in any other acute disease. The loss usually is from 200,000 to 1,000,000 cells per week but Grawitz mentions a case of acute streptococcus septicemia with hemorrhages in which the red cells fell in 24 hours from about normal to 300,000. The qualitative changes of the blood are marked; degenerations, poikilocytosis, polychromatophilia, etc. Nucleated reds seldom are present in large numbers. The leucocytes vary as the patient's resistance. In some cases the counts are very high, in other cases even subnormal.

We have had 26 cases of well-marked septicemia. The drop in the count between admission and death was from 900,000 to 1,600,000 cells. The final red counts in 15 fatal cases were: from 1,000,000 to 2,000,000, 2; from 2,000,000 to 3,000,000, 3; from 3,000,000 to 4,000,000, 4; and above 4,000,000, 6.

It is thus seen that there are 2 groups of cases, those with high and those with low counts, and that the infection is not serious because of the anemia alone.

In 4 cases there was no leucocytosis at death. In 21 cases the leucocytes varied from 11,000 to 47,000. These cases showed great variations in the blood formula. In 1 case with 8000 leucocytes 96.6% and in another with 10,400 leucocytes 92.6% were polymorphonuclears. In a case of gonorrheal septicemia the red count at death was 2,318,000, hemoglobin 30% and the leucocytes 47,000.

Chronic Septicemia of cryptic origin often would pass unrecognized were it not for the anemia. Cases of chronic abscess formation may have but little anemia. In Ewing's case of empyema the red blood cells, after a duration of 1 year, numbered 1,800,000 and the hemoglobin 25%. In cases of pelvic abscess of even 2 years' duration only a slight anemia may develop.

Blood in Endocarditis.—In simple (rheumatic) endocarditis the blood cultures are sterile in 90% of the cases (some would exclude from this group all in which the cultures are not sterile) and cultures from the vegetations made at autopsy show no constant organism.[138]

Instead of the terms "ulcerative," "malignant," or "infectious" endocarditis, Libman [139] suggests the term "bacterial" endocarditis and if the causal organism is known he substitutes the name of this for the word "bacterial" (*e. g.*, acute or subacute streptococcus endocarditis, etc).

In all cases of septicemia we may expect to find the heart valves involved. Especially is this true of streptococcus, pneumococcus, gonococcus, meningococcus and influenza septicemias.

Subacute streptococcus endocarditis, a condition usually fatal, is a disease of definite bacteriology due to a constant individual type of streptococcus which belongs to the saprophytic group, is of low virulence, is non-hemolytic and may (viridens) or may not (saprophyticus) be green producers. Biologic and immunologic tests, however, fail to show any constant identity between the individual streptococci concerned in producing this disease.[140] Libman especially has described this type under the name subacute bacterial endocarditis due to streptococcus viridens. Swift and Kinsella (*ibid.*, p. 381) warn us not to include in this group all cases with endocarditis from whose blood viridens may be cultivated since other characteristic signs as petechiae, embolic lesions and a progressive downward course are also necessary for that diagnosis.

Typhus Fever.—We have studied the records of but 4 cases of typhus fever:

Case I.—Male of 36 years. On admission: red cells 5,400,000, hemoglobin 72% and leucocytes 18,600. The temperature varied from 103° to 104° F. On the eighth day after admission the white count was 25,400; the temperature had then begun to fall. Five days later, the temperature then normal, the total count was 24,300; s.m.3.2%, l.m. and tr. 6.6%, pmn. n. 90% and eos. 0.2%.

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[138] Swift and Kinsella, Arch. of Int. Med., Mch., 1917, xix, p. 381.
[139] Am. Jour. Med. Sc., 1912, vol. 144, p. 313.
[140] Kinsella, Arch. of Int. Med., Mch., 1917, xix, p. 367.

Case II.—Male of 19 years. On admission: red cells 4,500,000, hemoglobin 70% and leucocytes 8600. This count remained normal for 4 days during which time the temperature varied from 102° to 105° F. On the fifth day the count was 12,500 and on the tenth day, with temperature normal, the total count was 10,000, s.m. 6%, l.m. and tr. 4.2%, pmn. n. 89.4% and eos. 0.2%.

Case III.—Male of 30 years. On admission: the red cells 5,500,000, hemoglobin 85%, and leucocytes 7000. They remained normal 3 days during which time the temperature ranged from 101° to 103° F. On the fifth day, the day of death, with temperature 98° F., this count was 38,000, s.m. 5.8%, l.m. and tr. 1.2% and pmn. n. 95%.

Case IV.—Male of 22 years. On admission: red cells 5,400,000, hemoglobin 85% and leucocytes 9200. This count remained normal for 3 days during which the temperature varied from 102° to 104° F. On the tenth day the leucocyte count had risen to 11,600 but the temperature had already been normal 3 days. On the twelfth day the leucocyte count was normal. On the ninth day the leucocyte count was 10,800, s.m. 15%, l.m. and tr. 11.5%, pmn. n. 72%, eos. 1.0 and Mastzellen 0.8%.

From these 4 cases (daily counts were made in all) it is seen that the leucocytes are low, even normal, on admission, then when the temperature has begun to fall or is already normal they rise to a maximum and then they fall to normal. (Compare with influenza and variola.)

Ewing and Thomas report an absence of leucocytosis in typhus fever.

Measles and **German measles** have almost no influence on the red blood-cells and cause no, or only a slight, leucocytosis but more often a leucopenia (see page 521). In the post-febrile stage the large mononuclears are increased.

Plantenga found in the 13 cases of measles and the 9 of Rötheln which he studied a neutrophile hyperleucocytosis of even 20,000 cells during the prodromal stage. During the eruptive stage this rapidly gave place to a hypoleucocytosis, due to the disappearance of the neutrophile cells, with sometimes a lymphocytosis and the disappearance of eosinophiles.

Renaud found in 6 cases that this preliminary leucocytosis reached its maximum about 6 days before the rash appeared. This permits one to isolate a suspected case early.

Tileston could not confirm this leucocytosis during the prodromal stage, and thought that all leucocytoses could be attributed to complications.

We have very little material, but of 9 recent cases in only 1 was the count above 8600 during the height of the fever (17,200).

Scarlet fever causes a slight anemia, the count averaging 4,500,000 (Reckzeh), and a leucocytosis which develops even 6 days before the rash appears and continues for even 12 days into the convalescence after the temperature has reached normal. Scarlet fever furnishes an interesting exception to the general rule that the leucocyte count runs roughly parallel to the temperature. The leucocyte counts vary from about 10,000 to 40,000 (in mild cases, 10,000 to 20,000; in moderate, 20,000 to 30,000, and in severe 30,000 to 40,000) according to the severity of the case and its duration.

The neutrophile cells are relatively increased (to from 85 to 98%, highest in the fatal cases). There is often an early eosinophilia, which is important in the diagnosis since it excludes various septic conditions, which reaches the maximum 2 or 3 days after the rash appears and disappears with the leucocytosis. (In other diseases these cells disappear during the fever and return with improvement.) Their failure to reappear is considered a bad sign.

Diphtheria.—In diphtheria a moderate anemia develops which amounts to a loss of about 2,000,000 cells at the time of defervescence. During the height of the disease, however, the red cell count and the specific gravity of the blood often rise. (The injection of these bacilli or their toxins into the circulation of animals has a lymphagogue action which results in a hypercythemia.) This hypercythemia occurs most commonly in this of all the acute infections. In Cutter's case the cells varied from 7,200,000 to 7,800,000; in Morse's from 5,000,000 to 5,500,000 during the first, and reached 6,800,000 during the second week. With the drop in the count nucleated reds and polychromatophilic cells appear. A slight leucocytosis of from 10,000 to 15,000, due to an increase of the polymorphonuclear neutrophile cells, is the rule but in severe cases the count may reach 17,000 and with complications even 30,000. In some fatal cases there is leucopenia. Myelocytes, even from 3 to 16%, are often found especially in the fatal cases. Morse says: "The examination of the blood in diphtheria is of no practical clinical importance in diagnosis, prognosis, or treatment."

In ordinary **follicular tonsillitis** the counts are often as high as in diphtheria.

Smallpox.—"No other disease is so destructive to the red blood-cells." (Hayem). A low red cell count is the rule as the temperature falls and yet this may be due in part to a dilution of the blood plasma resulting from the relaxed vasomotor tone which obtains then. Pick and Weil say that there is anemia in the severe, but none in the mild, cases. During the pustular stage of severe cases, however, there may be a true loss of 2,000,000 cells while in the hemorrhagic type the anemia is severe. Regeneration is slow, lasting about 14 days. Nucleated reds (normoblasts) are rare, except in the hemorrhagic cases in which they may be very numerous.

From the onset the total leucocyte count is normal but the blood formula is very characteristic in this disease. The polymorphonuclear neutrophiles are decreased, averaging about 40%, but they may sink to 20 or even to 14%. The small mononuclears vary from 30 to 40%, the large mononuclears from 4 to 10% and the myelocytes and irritation forms each 2 to 10%.

Smallpox itself causes no leucocytosis, and yet during the pustular stage a leucocytosis is often present.

Tuberculosis.—Tuberculosis is a disease which is accompanied by the greatest variety of blood pictures. In a few cases there is anemia of the highest grade (*e. g.*, v. Limbeck's case of tuberculosis of the peritoneum and abdominal organs, with a red blood-count of 730,000 and hemoglobin 25%;

such cases are so rare that this one is doubted by Cabot); in others, one of moderate grade, and often more apparent than real, while in others there is none.

In tuberculosis in general a mild grade of chlorotic anemia is the rule. This "pseudochlorosis tuberculosa" occurs in cases with slight involvement of the apex ("anemia of onset") without fever and in tuberculosis of bones and lymph-glands. The count is almost normal, the leucocytes normal and the hemoglobin somewhat reduced. In other cases there is a lymphocytosis, absolute or relative, while in some few cases there is a reduction of the count as well as of the hemoglobin. Qualitatively some of the red blood-cells (not the majority, as in chlorosis) are rather pale and small. Poikilocytes while usually few may be numerous, but not as numerous as in other cachexias of the same degree. Maragliano's endoglobular degenerations are seen in severe cases, especially in those with mixed infections. Nucleated reds are rarely present even after a severe hemorrhage causing extreme anemia. This helps in the differential diagnosis between tuberculosis and carcinoma.

Cabot who believes that the tuberculous virus has but little effect on the blood thinks that the above-mentioned changes are due to secondary infections or to drains upon the proteid of the blood from diarrhea, effusions, starvation, prolonged suppuration, etc., and Miller, Lupton and Brown[141] report remarkably few blood changes in cases of pulmonary tuberculosis undergoing sanitarium and tuberculin treatment.

In tuberculosis without secondary infection, with the possible exception of meningitis, the leucocytes are not affected. This is important in the diagnosis of tuberculous peritonitis, osteitis and acute miliary tuberculosis. There also are no important qualitative changes, for the lymphocytosis with a normal count, so often mentioned, is common to all cachexia-producing conditions. If a leucocytosis does develop it is of the ordinary inflammatory type. The eosinophile cells are increased in some cases with cavity formation. Since a similar increase follows the injection of tuberculin some think it is due to auto-intoxication from the cavity. Myelocytes appear in the peripheral blood of advanced cases.

Chronic Pulmonary Tuberculosis.—Grawitz has divided cases of chronic pulmonary tuberculosis into three groups: Group 1, with slight involvement of the apex and without fever. This is accompanied by the pseudochlorosis tuberculosa (see above) and a normal leucocyte count. Some early cases have practically normal blood. Group 2, of cases of chronic phthisis with cavity formation but without other complication and with slight temperature, is noteworthy since the blood picture is practically normal as regards count, hemoglobin, specific gravity and dry constituents. This is remarkable since during the cavity formation there is general emaciation. The leucocytes are normal or slightly increased, from 10,000 to 15,000 per c.mm. These patients earlier may have had a distinct chlorosis. Group 3 includes cases with hectic fever (supposed by many to be due to a secondary infection but by others to be due to a pure infection with the tubercle bacillus) in which often a true anemia develops, with evidence of blood destruction, which may progress until death. The drop of the count in these cases may be very rapid.

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[141] Am. J. of Med. Sc., May, 1912, No. 5, cxiii, p. 683.

In a recent case of pulmonary tuberculosis 2 days before death the red count was 1,473,000, the hemoglobin 15% and the leucocyte count 9000 (pmn. n. 88%, s.m. 5.9%, l.m. and tr. 4.7% and eos. 0.35%. There were also 4 normoblasts and 4 megaloblasts per 1000 leucocytes).

In chronic tuberculosis a leucocytosis is the rule, especially if a secondary infection is present as usually is the case. Von Limbeck considers the presence of a leucocytosis proof that there is a secondary infection. Others disagree since in the chronic septicemia form, which is a pure infection, there usually is a slight leucocytosis and in caseous pneumonia (a pure infection) the leucocytosis may be as high as in croupous pneumonia.

The normal count of the second stage has aroused considerable speculation. Some believe that potentially there must be an anemia but that this may be masked in some cases by the concentration of the blood due to sweating, diarrhea, vomiting or by a dyspnea which always tends to raise the count. Others think that there is an anemia which is covered by an oligemia and autopsies on patients with this stage of tuberculosis do suggest this. Von Limbeck claims that there is an oligemia due to a changed water metabolism which results in a general drying of the tissues and that this concentrates the blood. Grawitz says it is due to the lymphagogue effect of the products of caseous nodules.

After hemoptysis the regeneration of the blood may be rapid (see page 595). If *after an operation* on a tuberculous focus the hemoglobin does not rise rapidly the operation was probably incomplete. In some cases an anemia indicates an advance of the disease while in others the count may rise without any corresponding change in the condition of the patient. In *fibroid phthisis* there is as a rule no leucocytosis while in *acute phthisis* the anemia may be pronounced and progressive. In cases of *cavity formation* there usually is a leucocytosis. Some cases of *extensive tuberculous pneumonia* have little leucocytosis; others, as high an one as croupous pneumonia. In *acute miliary tuberculosis* there usually is no change in the red blood-cells, hemoglobin or leucocytes but a few cases were reported with a very low white-count, even from 500 to 600 cells, over 90% of which are polymorphonuclear neutrophiles.

Tuberculosis of the serous membranes is accompanied by a mild secondary anemia (unless the blood be concentrated by diarrhea) without a leucocytosis except in meningitis, in which a leucocytosis is the rule (Osler). In *tuberculosis of the lymph-glands* there is no leucocytosis, more often a leucopenia, until caseation begins.

The *injection of tuberculin* into a tuberculous patient sometimes causes a leucocytosis with a rise of eosinophiles. In tuberculosis of the bones there is marked absence of leucocytosis until a secondary infection develops; a high white-count indicates acute abscess formation, but after the abscess has persisted for some time the count may remain normal until a secondary infection develops. In bone cases the red counts are rarely diminished, but the hemoglobin is low.

That the anemia found in *children* should be so slight is rather remarkable since their blood usually is so susceptible to infection. Brown[142] in 73 cases in very young persons found the red blood-cells diminished only in the long-standing extensive cases, but the hemoglobin was diminished somewhat in all.

Of 17 cases of *acute miliary tuberculosis* in 5 the red cells stood between 3,600,000 and 4,000,000 and in 6 over 5,000,000. The color-index was quite low, in ½ the cases from 0.4 to 0.6. The leucocytes varied from 1000 to 9000, the majority (9) from 3000 to 6000. One case had an interesting differential count (total, 3500; s.m. 6.5%, l.m. 10.8%, pmn. n. 81.9%, eos. 0.5%). Warthin's case with a lower count, had 91.48% pmn. n.

Warthin reported a case with the leucocytes below 2,000, on 1 day (with a chill) 600, and Cabot a case with 550 white cells per 1 c.mm.

In miliary tuberculosis, if in any condition, one would expect to find Bacillus tuberculosis in the blood stream (see page 29). How difficult this is, is shown by Wilson[143] in Warthin's laboratory who calculated from a study of the post-mortem clots from the heart that the heart blood at death contained no more than 40 individual bacilli. Dieterle, (*ibid.*) in that same laboratory, studying a case of acute miliary tuberculosis complicating chronic leukemia found one bacillus to each 15 slides of blood from the right heart.

Secondary infections of the blood stream, however, are common in tuberculosis and increase in frequency as the disease advances.[144] Far-advanced cases are 2½ times as likely to have them (61%) as are moderately advanced cases (24%) and these latter are 2½ times as likely as are incipient cases (9%). Febrile cases are 6 or 7 times as likely to have secondary organisms in the blood as afebrile cases, and open cases (40%) are 8 times as apt as closed cases (5%) to show organisms in the blood.

Tuberculous Meningitis presents an illustration of the general rule that the tendency of an infection to cause a leucocytosis depends on the location of the lesion as well as on the organism, for tuberculosis of the meninges usually causes a leucocytosis.

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[142] Trans. Med. Soc. of the State of California, 1897, p. 168.

[143] Jour. of Infect. Dis., Aug., 1916, Vol. 19, p. 260.

[144] Brown, Heise and Petroff, Tr. of the Ninth Annual Meeting of the Assoc. for the Study and Prevention of Tuberculosis.

In only 3 of 15 cases was the count below 10,000. One of these was a case of acute general miliary infection and in the other 2 but 1 count was made. The highest was 26,800. The leucocyte curve is a very irregular one, 3 of our cases with the high counts had also periods with low counts.

In the series of 43 cases reported by Cabot there was a leucocytosis in 32.

Tuberculous Peritonitis.—Of 19 cases of tuberculous peritonitis, 7 had counts between 3,000,000 and 4,000,000 and 6 between 4,000,000 and 5,000 000. The color-index varied from 0.5 to 1. Of 22 cases there was a leucocytosis in 9 (highest count, 22,400). Of Cabot's 60 cases there was a leucocytosis in 14.

Tuberculosis of Bones and Joints.—Of 15 cases of joint and bone tuberculosis in the Johns Hopkins surgical clinic, 6 had a leucocytosis.

It is believed that during the process of abscess formation a leucocytosis is the rule and that in time this will disappear to reappear in greater degree if a secondary infection develops. This may explain the high count following operation. This leucocytosis soon subsides and so long as the abscess drains freely will not reappear.

Tuberculosis of the Intestine.—Of 5 cases of intestinal tuberculosis there was a secondary anemia in 2 (3,000,000 and 2,800,000 red cells) and in one a leucocytosis of 14,000 which disappeared soon after admission.

Two cases of *Renal Tuberculosis* showed no leucocytosis.

In one case of *Addison's Disease* the red cell-count was 6,000,000, hemoglobin 92% and leucocytes 9000. The rule in this disease is a marked anemia.

Typhoid Fever.—For the *bacteriology* and *serology* of typhoid fever see pages 562 and 564. In the fresh blood smear in typhoid fever may sometimes be found the very large phagocytic cells crowded with red corpuscles which Mallory described. Thayer[145] who reported the cases of the Johns Hopkins clinic found that from the end of the first week until defervescence there was a gradual reduction in the number of *red cells* and that regeneration began with defervescence. In very long-continued cases the regeneration may begin slightly before the temperature reaches normal. The total loss of red cells averages 1,000,000 cells. The average count at the end of the third week, which is the usual limit of the disease, was 4,555,814. The loss may be accentuated during the fourth week and, indeed, the usual statement is that the anemia begins at this time. Transitory variations are common during the fever due to vomiting, sweating, diarrhea, etc. After a severe hemorrhage the anemia may be marked and regeneration begin at once.

Following some very severe cases a severe post-typhoid anemia may develop. In one case the red count was 1,426,000 during the fourth week; in another 1,300,000 during the third week (both Osler's cases) and in one 804,000 (Henry).

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[145] Johns Hopkins Hosp. Rep., vol. viii.

In one case, with distinct invasion of the bone-marrow by Bacillus typhosis, the red cell-count in 7 days fell from 3,752,000 to 1,006,000, the leucocytes rose from 4,200 to 33,300 while 26,000 nucleated reds per c.m.m. appeared of which 2,880 were megaloblasts and intermediates.

Usually there are no qualitative changes. After a hemorrhage nucleated reds are sometimes seen.

There is always a more marked reduction in the hemoglobin than in the red cell-count and it returns to normal more slowly than do they.

Some think that the *leucocytes* are slightly increased at the very onset of an attack of typhoid fever but most observers find them subnormal during the whole course. Certainly their count diminishes gradually from the end of the first (when the average count is 5400) to the fifth week, at which time the average of Thayer's cases was 5386. Some cases reach 2,000, others 1,000 per c.mm., some even lower. Thayer found no cases with an initial leucocytosis. The longer and more intense the infection the lower the leucocyte count. In a few cases without complication the count is above 10,000 throughout the whole course. Temporary variations are common, the count rising, *e. g.*, to 10,000 cells after a cold bath, yet with the differential count unchanged.

The *differential count* for the first 5 weeks shows a progressive decrease in the polymorphonuclear neutrophiles, usually to 60%, often below 50%. There is also an increase of the endothelial cells. These are especially numerous at the height of the fever. The eosinophile cells are below 1% as a rule until convalescence, when they increase even to an eosinophilia. They may, however, in long continued cases, rise with the increase in the red cells before the temperature is normal. During convalescence the count slowly rises, but the blood retains its characteristic features for about 3 weeks after the temperature is normal.

The blood picture may be modified by *various complications*. Hemorrhage causes an acute post-hemorrhagic anemia with leucocytosis. The lowest count of Thayer's series was 1,992,000 cells. Regeneration begins at once.

The inflammatory complications cause a rise of the count of white cells, even a true leucocytosis. This is true of furunculosis, phlebitis, thrombosis, bronchitis, periostitis, pleurisy, pneumonia, etc. A definite rise of a count already very much reduced is for that person often a true leucocytosis; for instance, one case of typhoid fever with a leucocyte count of 1600 developed parotitis whereupon the count rose to 3200 cells, a reaction comparable in a normal person to a rise to about 15,000 cells. In 1 case with empyema the count was 44,500; in a second case of empyema due to Bacillus typhosus the count was 23,000 cells of which 68.5% were polymorphonuclear neutrophiles, 12.7% small mononuclears and 17% large mononuclears.

Three of 5 cases of pneumonia, of whom 2 died, had counts above 10,000 cells and 2 cases, both of whom died, had counts below 10,000 cells. The counts in all of these cases had a rather small percentage of polymorphonuclear neutrophiles. In 1 case of periostitis due to Bacillus typhosus the leucocytes were 18,000 of which 72.5 % were polymorphonuclear neutrophiles. Thayer cites many similar illustrations showing that in typhoid fever the leucocyte reaction in cases with complications depends much upon the tissue infected and that there is a tendency, in cases with a leucocytosis for the formula of typhoid fever to persist.

In all cases of suspected *intestinal perforation* the leucocytes should, from the first, be followed with the greatest care. In most cases the leucocytes rise either to an absolute leucocytosis of 10,000 or over, or to one relative to the previous counts. Then the count sometimes drops coincident, perhaps, with the spread of the peritonitis. In some fulminant cases the count falls from the first. In the so-called pre-perforative stage there is a slight leucocytosis due to the local peritonitis. While the leucocyte curve has but little absolute value, yet it has much when interpreted in the light of the physical examination. If the abdominal signs are suggestive of perforation the operation is performed whatever the leucocytes may show. But if the local abdominal signs alone seem hardly sufficient to justify operation a rising count would settle the question. Of course a rising leucocytosis might mean something other than perforation. In one of our cases it meant appendicitis. Every case in which there is very good reason to fear perforation is operated upon, under the belief that a quite unnecessary operation will be of benefit since if perforation has occurred from 30 to 50% will be saved and if there is no perforation the course of the fever will at least probably be milder after the operation.

Pneumonia.—In acute lobar pneumonia the coagulation of the blood is, as a rule, rapid. The count of the red blood-cells is normal during the fever, but there may at first be a rise, as in Sadtler's case to 7,000,000. After the crisis there is always a drop of about 500,000 cells and sometimes a slight post-febrile anemia. The hypercythemia is probably due to a concentration of the blood which may cover a real anemia caused by the loss of blood in the exudate and by the destruction of the blood-cells, as shown by the jaundice and the urobilinuria. Some cases have a true and severe anemia with a loss of about 2,000,000 cells. On the day of crisis there is a drop due in part to a general peripheral relaxation (Grawitz) and partly to a true anemia which up to this time has been covered by the hypercythemia.

Nucleated reds are more common in the blood in pneumonia than in other acute fevers. Both normoblasts and megaloblasts may be present. The latter have a bad prognostic import only when present in considerable numbers. It is thought that at the time of the crisis the cells crenate more readily than normal.

In 34 cases of acute lobar pneumonia studied with special reference to the red blood-cells there was a drop in the count during the lysis or just after the crisis, generally of about 1,000,000 but in some cases of 2,000,000 cells, which usually only restored the count to that level which obtained before the hypercythemia. The later counts showed small gains and losses in an even number of cases and of about the same degree, but in 9 cases there was a permanent loss of from 900,000 to 1,500,000 and in 4 cases a gain of from 700,000 to 1,900,000 cells.

In pneumonia the inflammatory leucocytosis has been best studied. None of our cases showed an initial hypoleucocytosis, as claimed by Pick. From the first, *i. e.*, from 6 or 8 hours after the chill, the leucocytes are increased. The maximum count just precedes defervescence and the drop immediately follows this. This leucocytosis is an expression of the resistance of the patient to the infection and depends but little on the fever or on the extent of consolidation. Cabot has divided the cases into 3 groups: (1) Those with good resistance and a mild infection, in which there need be no leucocytosis; these cases all recover. (2) Those with a severe infection and a good resistance, in which the leucocytosis is high, between 20,000 and 30,000, but in some cases over 100,000 and even 115,000 (Lohr). This group includes about 90% of all cases. (3) Those cases in which the infection is severe and the resistance poor. In these there is no leucocytosis or even a fall. These cases are usually always fatal. This last group includes the terminal pneumonias of chronic diseases, the pneumonia in the aged, etc. In fatal cases the percentage of polymorphonuclears may rise considerably although the total count may not at all.

The statement is made that the leucocytes do not drop with a pseudocrisis and even rise. The fall in the leucocytes begins just before, just after or with that of the temperature. In cases ending by crisis the count falls by lysis reaching normal on about the second day, while if the temperature falls by lysis the leucocytes fall still more slowly. If a slight temperature persists after the crisis the leucocytes remain elevated until the temperature is normal. In fatal cases there is often an ante-mortem rise of the white cell count. If there is delayed resolution the leucocytes may stay elevated even for weeks and then slowly drop with the temperature. For the count to remain elevated after a supposed lysis suggests delayed resolution, empyema, or pulmonary gangrene.

A high leucocyte count has no value in prognosis; it merely means that the patient is making a vigorous fight.

In the Johns Hopkins Hospital the leucocytes of pneumonia cases were counted twice daily. We [146] have compiled the records of 158 uncomplicated cases with recovery, 56 uncomplicated cases with death and of 80 cases with various complications. In the uncomplicated cases with recovery the degree of the leucocytosis bore no relation to the extent of the consolidation. In 38% of the uncomplicated cases with recovery the count was below 20,000 and in 7% above 40,000. Age had little influence on the leucocyte reaction since exactly the same percentage of cases below 40 years of age had a leucocyte count below 20,000 as of those older.

Of the 77 cases who terminated by crises the leucocyte counts of those above 40 years of age were somewhat higher than of those younger. In 18% of these cases the counts varied from 10,000 to 15,000. These, clinically, were very mild cases. In 25% they varied from 15,000 to 20,000, and in 8% were over 40,000. There was a sharp precritical rise in 42% of these cases.

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[146] Johns Hopkins Hospital Reports, 1910, Vol. XV.

Of 81 cases with lysis the count during the course kept below 10,000 in 2%, from 10,000 to 15,000 in 20%, from 15,000 to 20,000 in 14%, and above 40,000 in 10%. There was a sharp rise just at lysis in 34% of these cases.

Those rises which occurred just before lysis or crisis amounted to from 5000 to 10,000 cells as a rule, but in a few it was over 20,000, and in 1 case 30,000. The highest count seen in a case was 105,500 in a young man 25 years old who recovered.

Of the cases with crisis the fall in the leucocytes preceded the drop of temperature in 15%, accompanied it in 41% and followed it in 44%. In the cases ending by lysis the drop began before that of the temperature in 18%, with it in 43%, and followed it in 39%. (Notice the similarity in these figures.) For the leucocyte count to reach normal required, in the cases with crisis, from 1 to 20 days; mean, 3 days.

A well-marked pseudocrisis occurred in 9 cases. Of these 2 were accompanied by a rise of leucocytes, 4 by a fall, and 3 by no change in the white cell count. In cases in which a slight fever continued after its drop the leucocyte count remained from 12,000 to 15,000 until the temperature reached normal.

There were, in this series, 56 fatal cases. The leucocyte counts in these were almost the same for the various decades of age as in those with recovery. During the course they remained below 10,000 cells in 23 of the cases (in 1 case they reached even 1700); from 10,000 to 15,000 in 23%, from 15,000 to 20,000 in 15% and over 40,000 in 1 case. At the time of death the count was below 10,000 in 17%, from 10,000 to 15,000 in 25%, 15,000 to 20,000 in 10% and above 40,000 in 8%. All of the last cases were under 30 years of age. Toward death there was in 70% a progressive rise and in 30% a fall.

The absence of a leucocytosis does not necessarily indicate a fatal outcome. In 1 case with extreme toxemia and a count of 8000 the leucocytes slowly rose to 14,000 as the patient recovered.

Daily Variations in the Count.—Counts made in the forenoon and afternoon, separated by an interval of about 9 hours, differed by from 1000 to 26,000 cells (as a rule from 4000 to 6000; mean, 4000). There was no difference in these variations before and after the crisis. While the temperature is fairly constant the counts vary less and yet there was in general no parallelism between fluctuations of temperature and of leucocytes.

In cases of delayed resolution the leucocytes reached normal before, with, or after the temperature. In some cases both temperature and leucocytes were normal before resolution was complete.

The cases of terminal pneumonia presented great variations. In our series there were 2 counts above 50,000 and 2 below 3500. Alcoholics had almost no leucocytosis and yet some recovered. In no cases followed by empyema did the leucocyte counts indicate the onset of this sequela. In 1 case of pneumonia followed by empyema the leucocytes did not rise at all until the empyema began while in another such case they had not risen up to the day of the operation. In 2 cases followed by pleurisy with effusion the leucocytes were normal after the crisis (6000 and 8000). In 3 fatal cases ending in abscess of the lung the leucocytes were respectively 46,000, 30,000 and 8500. In 35 cases with various pus infections, endocarditis, pericarditis, meningitis, parotitis, otitis media, phlebitis, thrombosis, tonsillitis, etc., very little could be learned from the leucocyte counts, *i.e.*, they did not change with the development of the complication.

Qualitative Changes.—The leucocytosis of pneumonia is of the polymorphonuclear neutrophile variety and yet the percentage of these cells is seldom 90%, often it is not over 80%. After the crisis it may drop to 60%

and even lower, due in part to an absolute increase of mononuclear non-granular cells. The eosinophile cells may disappear from the peripheral circulation during the attack and reappear at the crisis, at which time also myelocytes, even 12% of the total white cell-count, may appear. The large basophile mononuclears also may be increased. For the percentage of polymorphonuclear neutrophile cells to be above 90 or below 50 is thought to indicate a bad prognosis.

Glycogen can usually be demonstrated in the leucocytes, in amounts varying with the temperature and the extent of consolidation.

The platelets may even disappear during the fastigium but after the crisis they return and increase to above normal.

The fibrin network is much increased. Coagulation is rapid. The specific gravity of the blood varies as the count and is high. The toxicity of the blood is even doubled.

In a doubtful case a high leucocytosis would exclude malaria and typhoid fever and suggest a central pneumonia. This is especially important in the very old and in the very young patients.

In the **bronchopneumonia of influenza** of the epidemics of past years there has been a polymorphonuclear neutrophile leucocytosis of from 10,000 to 15,000, and in severe cases of from 20,000 to 25,000 cells per c.mm.[147] but during the epidemics of 1917-20 it was more common to find a leucopenia of under 5000, *i. e.*, 2800 or below, even in cases which develop an empyema. In one of our cases it was 83,400.

In **acute epidemic cerebrospinal meningitis** there is a leucocytosis (see page 513) but this disease is not primarily an inflammation of the meninges but is a septicemia in which the bacteria tend to become localized in the membranes of the brain and cord. This is what might be expected from the clinical picture, especially that of the fulminating cases. That the bacteria must frequently be present in the blood in large numbers and at a very early stage is suggested by the very early appearance of the petechial eruption (hence the term "spotted fever").

In cases of infection by **intestinal parasites** a slight leucocytosis is the rule. In 12 of our 18 recent cases these cells varied from 11,200 to 34,000. In 4 cases with fever the counts were normal.

Bronchial Asthma.—In bronchial asthma the most interesting find is an eosinophilia of even 53.6%. This is important in diagnosis and also as a means of predicting oncoming paroxysms (see page 522).

Of 17 cases, the red cell count was over 5,500,000 in 7 and the lowest count was 4,900,000. There was a leucocytosis of from 10,000 to 15,700 in 6 cases. Of 8 cases in which differential counts were made 6 had an eosinophilia. (The absolute numbers of eosinophiles in these cases were 728, 712, 535, 856, 702 and 1720 [20% of 8600 leucocytes per 1 cmm.]).

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[147] Davis, Arch. Int. Med., 1908, vol. ii, p. 124.

Acute Rheumatic Fever.—"The blood is the best index of the severity of this disease" (acute rheumatic fever) (Osler). Its virus causes rapid destruction of the red cells, lowering the count often (but not always) from 1,000,000 to 2,000,000 cells. The high counts sometimes seen during the attack may be due to the profuse sweats. The anemia is most evident at the time of convalescence. Hayem, Turk and others say that the count is lowest at the height of the fever and that regeneration begins at once with defervescence. Nucleated reds are seldom present. In no other disease is the fibrin network so thick.

There is a leucocytosis as a rule, the count running parallel to the severity and acuteness of the disease. Cabot's average was 16,000. The blood formula is that of an acute inflammatory disease.

Of 77 cases of the Johns Hopkins clinic the red cell-counts varied from 2,000,000 to 3,000,000 in 3, from 3,000,000 to 4,000,000 in 15, from 4,000,000 to 5,000,000 in 45 and 5,000,000 and over in 14 cases. The mean count was 4,500,000. In these cases therefore there was very little anemia. Of 81 cases, the leucocytes were below 5000 in 1 case, from 5000 to 10,000 in 23, 10,000 to 15,000 in 36, 15,000 to 20,000 in 15 and above 20,000 in 6 cases.

One case, a man 56 years of age, was admitted with red cells 1,720,000, hemoglobin 27% and leucocytes 12,400. He gave the history of painful, swollen, red joints 4 weeks before. He recovered rapidly.

The bacteriological study of the blood has failed to throw any new light on the etiology of this disease and the opinion is still held by many that if any organism can be cultivated from blood or joint the case is one of acute infectious arthritis and not of acute rheumatic fever (acute articular rheumatism). One of the last good studies of this condition is that of Swift and Kinsella,[148] who found no type of streptococcus constantly or frequently (*i. e.*, not over 10%) associated with this disease and those which were found varied so much that none deserved the name Streptococcus rheumaticus.

In SUBACUTE OR CHRONIC RHEUMATISM there is no leucocytosis.

Arthritis Deformans. Subacute Infectious Arthritis.—McCrae found that in 33 cases of arthritis deformans the average of hemoglobin was 70.6%, of the red cells (in 29 cases) 4,468,000 and of the leucocytes, 7600. The differential counts were normal.

Appendicitis.—In cases suggesting acute appendicitis the leucocytes are counted each hour. If with suggestive history and symptoms there is a rising leucocytosis an operation is performed without delay, while if the abdominal signs are marked the operation is performed whatever the leucocyte count may be. If the leucocyte counts are stationary, even though high, when the patient is first seen, one may wait; but if rising even slightly there should be no delay. The count may be normal in mild or very severe cases, or in cases with well walled abscess. A leucocytosis of 20,000 or above indicates acute appendicitis, probably an appendix full of pus and quite tense. This will fall after the appendix ruptures. (At least those cases admitted soon after the appendix had ruptured often have low or even subnormal counts even though the peritonitis is spreading.) In appendicitis a count above 15,000 means an active process. Over 20,000 is a high count and means pus, gangrene, or peritonitis. Fulminating cases may die without any sign of reaction on the part of the white cells.

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[148] Arch. of Int. Med., Mch., 1917, xix, p. 381.

In CHRONIC APPENDICITIS with abscess a stationary leucocytosis means that the latter is well walled off. If the abscess has been present for some time the count is seldom above 12,000 and usually is nearer 7000. If now one operates the count will at once rise to 20,000 or over and then gradually drop. This is perhaps due to the exposure of new tissue to infection or to absorption. If the count after the operation remains high it means that a pus pocket is still unopened. In cases with well-walled abscess and a normal leucocyte count the leucocytes may fluctuate markedly. For this no explanation can yet be offered. In cases with a spreading peritonitis the count of white cells may rise, or drop and then rise, or may fall even to subnormal. The falling leucocytosis is a worse sign than a high stationary count.[149]

In CHRONIC OBLITERATIVE APPENDICITIS and SUBACUTE APPENDICITIS WITHOUT EXUDATE there is no leucocytosis.

The red cells are not affected in cases of appendicitis unless there is a long-standing abscess, then there may be a secondary anemia. Da Costa mentions an early slight anemia in most cases, in some a severe anemia.

ANEMIAS OF CHILDREN

The study of the blood of normal children is very important since they react to disease often differently from adults. We have even known a diagnosis of lymphatic leukemia suggested by a normal child's blood.

Children are much more susceptible to the agencies which produce anemia than are adults, their anemias develop more rapidly, become more severe and have a worse prognosis.

In the anemias of the very young a lymphocytosis is usually present and unripe elements soon appear, striking evidence of the activity of the bone-marrow. Among these are normoblasts, megaloblasts, myelocytes and large basophilic non-granular leucocytes which do not appear in normal blood. But these qualitative changes in the blood picture have less significance than in the adult and merely show the greater instability of the blood-regulating mechanism.

Certain cases of anemia are grouped under the term "anemia of growth" and are said to be due to the inability of the blood-building organs to keep pace with the increasing demands of the growing body. During this period wasting diseases, infections and any agencies deleterious to the blood, vascular system, or to the hematopoietic organs, such as poor food and bad hygienic surroundings, have a more pronounced effect than on the blood of an adult. This is well illustrated by the anemias of school children, which are ascribed by some to mental strain, lack of exercise, poor appetite, constipation, etc., but by others to bad tonsils, latent tuberculosis, etc. The development of the heart and blood vessels is closely related to that of the blood, hence chlorosis and other severe anemias of youth occur in association with hypoplasia of the cardiovascular system and are attributed to congenital defects.

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[149] See Bloodgood, Prog. Med., December, 1901.

A severe anemia in a child is perhaps never perfectly recovered from. Objective evidence of it may disappear and the child seem well, but relatively insignificant illnesses will bring it to light again.

THE ANEMIA PSEUDOLEUKEMICA INFANTUM of v. Jaksch was described as a severe anemia of young children, with the red cell-count usually from 1,500,000 to 3,500,000 but even as low as 820,000, a low color-index (0.50) and a leucocytosis of even 54,660 (in one case, 114,000) with a few myelocytes present. Many of the red cells are deformed and degenerated and many are nucleated. The leucocytes are characterized by their great variations in form, size and staining qualities. The platelets are increased in number. Cabot [150] thought that many different diseases are grouped under this diagnosis, including pernicious anemia, secondary anemia with leucocytosis (due especially to lues, rickets, etc.), Hodgkin's disease and even leukemia, all of which diseases are apt to be atypical in children.

MALARIA OF CHILDREN may cause a severe anemia with normoblasts and practically always megaloblasts present, but no marked leucocytosis unless it be due to an increase of the endothelial leucocytes.

CONGENITAL LUES causes the severest anemias. Many nucleated reds appear, the lymphocytes especially are much increased and the total white cell-count may reach even to from 50,000 to 100,000.

RICKETS causes a simple chlorotic anemia with a leucocytosis of even 30,000 which is in large degree a lymphocytosis.

A case of anemia in a 14-months-old child is entered on our records simply as "anemia with enlarged liver and spleen" (Osler). The red cells on admission numbered 1,252,000 per c.mm., the hemoglobin 20% and the leucocytes 14,700. The child was in the ward one month without improvement. The leucocytes varied from 13,000 to 26,500, always with the same formula (s.m. 40 to 52%, l.m. and tr. 5 to 18%, pmn. n., 38 to 62%, eos. 0 to 0.9%, neutroph. myeloc. 0.9 to 3% and Mastzellen 0 to 0.2%. The nucleated reds, which numbered from 24 to 250 per 1000 leucocytes, were chiefly normoblasts, some were intermediates and some megaloblasts and microblasts). Such a case resembles the French "splenomegalie chronique avec anémie et myélemie."

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[150] "Clinical Examination of the Blood," Fifth edition, p. 519.

Summer Diarrheas of Children.[151]—In some cases of severe diarrhea in children the red cell-count may rise even to 10,000,000 cells. The ordinary summer diarrheas are usually accompanied by a leucocytosis. In the simple dyspepsias the differential count of leucocytes is normal (total 13,500 to 36,000; s.m. 39%, l.m. and tr. 21.2%, pmn. n. 37.8% and eos. 2%), but in the more severe cases there is an increase in the polymorphonuclear neutrophiles (from 56 to 63%) and a decrease of the mononuclear cells (from 33 to 7%), the blood thus presenting the adult formula. In an acute intestinal toxemia and in the severe forms of enterocolitis a true leucocytosis is the rule, which is characterized by the presence of a few myelocytes and the absence of eosinophiles.

A leucocytosis with a wasting disease in a child usually indicates an inflammatory intestinal complication.

CHRONIC DISEASES

Chronic Nervous Diseases.—The blood in cases of chronic diseases of the nervous system presents nothing at all characteristic. Whatever changes are present depend on the general nutritional condition of the patient.

Such patients with Acute Chorea are very anemic, yet of 23 cases of the Baltimore Clinic this was true of but 3 and these 3 had heart complications. The lowest count was 3,400,000 reds. These cases sometimes have an eosinophilia of from 7 to 10% (Theleme).

In **general paresis**[152] Capp and Jenks found in some cases just before a paretic seizure an absolute leucocytosis, the increase affecting especially the large mononuclears. A slight anemia which progresses with the disease is the rule, except during the seizures when the count of red cells may temporarily rise.

In Maniacal Depressive Insanity [153] an anemia is the rule and almost always during the periods of excitement there is a leucocytosis.

In Acromegaly there is usually an increase in the count of red cells with eosinophilia and lymphocytosis (Ducati).

Diabetes Mellitus.—In diabetes mellitus one of the essential symptoms during the periods of glycosuria is the hyperglycemia of even 0.57% instead of, as normal, 0.1 to 0.2%. This hyperglycemia may cause a hydremia, *i. e.*, a dilution of the blood, and therefore a lowering of the count of red cells while the resulting diuresis tends to concentrate the blood. Later in the disease a cachexia with anemia develops which, however, may be well masked by the concentration of the blood. The leucocyte counts are normal. The patients often show a remarkable digestive leucocytosis.

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[151] Knox and Warfield, John Hopkins Hospital Bull., July, 1902.

[152] Am. Jour. of Insanity, January, 1900; Diefendorf, *loc. cit.*, 1903, vol. cxxvi.

[153] Fisher, Am. Jour. Insan., April, 1903.

Of 45 cases, the red cell-counts were below 4,000,000 in 3 cases (the lowest count was 2,000,000), from 4,000,000 to 5,000,000 in 13, from 5,000,000 to 6,000,000 in 10 and over 6,000,000 in 4. In 3 other cases the count at times was over 6,000,000.

Of 40 cases, the leucocytes varied from 5,000 to 10,000 in 25, from 10,000 to 20,000 in 7 and over 20,000 in 7. The highest leucocyte count was 44,000. These leucocytoses were due to pneumonia, septicemia, furunculosis, gangrene, etc. In a case of coma the leucocytes varied from 30,000 to 41,000.

Blood Lipoids.—"With an excess of fat diabetes begins and from an excess of fat diabetics die" is the very expressive way Joslin emphasizes the study of blood lipoids in diabetes. Lipemia (see page 556), or the presence in the blood of sufficient visible fat to give the plasma a milky appearance, is common in severe cases of diabetes on a fat-rich, carbohydrate-poor diet, but usually disappears after the diet is changed.

Basal Metabolism.—The conclusions of the accurate work of Joslin, Benedict and their co-workers on basal metabolism in diabetes may be summarized as follows:

While even in the same patient metabolism may at one time be increased and at another be below normal, yet severe cases of diabetes during the existence of an acidosis show an increase in metabolism of from 15 to 20%, the increased consumption of oxygen and the increased heat elimination running hand in hand. A similar increase is seen in normal persons in whom an acidosis is artificially induced. Following the disappearance of the acidosis the metabolism may become normal or below normal so that severe diabetics may live on less calories than the normal individual.

Bremer's blood-test for diabetes mellitus has, in some cases, proved of value. A thick smear of blood on a slide and a similar one of normal blood for a control are subjected to exactly the same treatment They are first heated to 135° C., then allowed to cool slowly and are then stained for 2 minutes with 1% aqueous Congo red solution. The diabetic blood will take a yellower stain than the normal. This test is said to be positive when the urine is sugar-free and even before sugar has ever appeared. Schneider found it positive in the cases of 2 normal men who were great meat-eaters and ascribed it to the reaction of the blood. Strauss confirms this opinion, finding it best in cases of acidosis. It is claimed to be sometimes present in leukemia, Hodgkin's disease and Graves' disease.

In this clinic a man was admitted during coma; no urine could be obtained by catheterization; the diagnosis of diabetic coma was made from this test alone and was confirmed later at autopsy.

Williamson's Test.—Twenty cubic centimeters of blood in a test tube are mixed with 1 c.c. of aqueous methylene blue (1 to 6000); 40 c.mm. of 60% KOH and 40 c.mm. of water are added. This mixture is allowed to stand for 3 or 4 minutes in boiling water. If the blood is diabetic it takes a yellow color.

Malignant Disease.—Malignant tumors are among the most important of anemia-producing diseases. They certainly produce a toxin which injures the blood, but the hemorrhages and the mechanical effects of the cancer on the gastro-intestinal functions must not be overlooked. And yet it is remarkable how long the blood will remain almost normal and then how rapidly cachexia and anemia will develop.

Cancers differ much in their effect on the blood. Some, for instance those of the skin or lip, may cause none or a slight secondary anemia, the so-called "pseudo-chlorosis carcinomatosa," while one of the stomach, "may give the perfect picture of primary pernicious anemia or, indeed, of leukemia." It is stated that the more malignant the tumor and the more extensive its metastases, the greater its influence upon the blood. But this certainly is not true. Our cases of huge tumors with rapidly spreading metastases had a slight chlorotic anemia while those which simulate pernicious anemia are more apt to be an insignificant-looking little nodule, usually on the stomach and discovered at autopsy. Those which cause the severest anemia are those which give rise to frequent hemorrhages, *e. g.*, those of the stomach and uterus and those also which mechanically disturb the functions of the digestive tract. In many cases with advanced cachexia, yet no hemorrhages, there may be even a rise in the red cell-count due to desiccation of the tissues (v.Limbeck).

The anemia due to cancer is as a rule of the secondary type and severer than that due to any other chronic disease. The first changes are in the size, shape and weight of the red blood-cells and later in the counts. As the cachexia develops the red cell-count may be as low as 2,500,000 cells or even 1,000,000 cells. An exception to this is in cancer of the esophagus in which cases the blood may be concentrated. Grawitz suspects that in some cases the anemia is in part only apparent, since the injection of carcinoma extract will produce a hydremia. There is a constant and often an early reduction in the hemoglobin. If it is normal the cell count will be found above normal. The average in long-standing cases is about 68.5%, in worse cases 57.5%, while the color-index averages about 0.65. This low color-index is an important diagnostic point between malignant and non-malignant tumors. Later, if the anemia assumes the pernicious type, the color-index may rise above 1. It has been claimed that after the removal of a cancer by operation the regeneration of the blood begins late and is never quite complete.

While the anemia is of the chlorotic type many or most of the red cells will be diminished in size while later, after it assumes the primary type, the cells will be large; yet the giant cells of pernicious anemia will be rarely seen except late, while microcytes will be numerous (Grawitz). The basophile granulation is very common. The deformities in size and shape and the degenerations of the erythrocytes may be absent or they may even be more marked than in tuberculosis, in which case this would be of diagnostic value. In any case, however, they will be a less prominent feature than in pernicious anemia. Nucleated reds are found even when the anemia is slight and in greater numbers than in the secondary anemies due to other causes. Their presence has a limited value in the differential diagnosis between cancer and ulcer of the stomach. There will be normoblasts as a rule although in those cases which simulate pernicious anemia a few megaloblasts also may be present. In cases with metastases to the bone-marrow their number may be surprisingly large.

There is a moderate leucocytosis in about 60% of all cases of malignant disease, an important point in the differentiation between benign and malignant tumors. This may be the first sign of a cachexia. Some cases suggest even a leukemia. In malignant esophageal stricture the starvation sometimes causes a leucopenia with relative lymphocytosis. Cancers of the uterus and stomach, so commonly accompanied by hemorrhage, usually cause a leucocytosis; and in malignant disease of the thyroid, pancreas and kidney the count sometimes is especially high. It is said that the faster the tumor grows the higher the leucocyte count, but there are many exceptions to this rule. Grawitz, who explains the leucocytosis as due to a lymphagogue action of the cancer extract which sweeps a great many leucocytes from the tissue spaces into the capillaries, considers that any leucocytosis is coincident with the softening of a tumor mass. After operation the leucocytes will drop, while a subsequent rise may indicate a recurrence of the disease even before it can be found physically.

The majority of leucocytoses due to malignant disease are of the polymorphonuclear neutrophile variety but some are lymphocytoses of even 43.7%. In other cases with a leucopenia of only 3,000 cells even 88.7% of these are polymorphonuclear neutrophiles. The eosinophiles are usually more in evidence than in other leucocytoses. Myelocytes are more numerous in the blood of cancer patients than in all other conditions except leukemia and pernicious anemia. It is said that a cancer may cause degenerative changes of the leucocytes before the quantitative changes begin.

The specific gravity of the blood is low. The plasma is rich in sugar, as rich even as in diabetes. Its alkalinity may be considerably decreased. The coagulability is normal or lessened unless sloughing or inflammation is present, in which case it may be rapid. The fibrin network usually is normal.

Cancers of the breast usually cause a slight leucocytosis (*e. g.*, leucocyte count 11,000). Cancers of bone often give a blood picture with many nucleated reds, both normoblasts and megaloblasts, and a leucocytosis with a high percentage of non-granular mononuclear cells and some myelocytes, but not as many coarsely granular cells as one might expect.

Cancer of the Stomach.—Cancer of the stomach usually causes a rather marked chlorotic anemia. Cabot found that of 129 cases, in 27 the red cell-count was above 5,000,000, in 26 below 3,000,000 and that the average of all cases on the first examination was 4,018,000. Of the 134 cases of the Johns Hopkins clinic, including those reported by Osler and McCrae, in 33 it was above 5,000,000 and in 16 below 3,000,000. The mean was about 4,000,000. The color index was always considerably below 1 unless the count was very low. Nucleated reds were rather rare. The count sometimes drops progressively till death (in one case to 1,786,000). High counts may sometimes be attributed to excessive vomiting. The differential diagnosis between cancer of the stomach and pernicious anemia is one of well-recognized difficulty and in many cases can be settled only at autopsy. The red cell-count may be as low as 500,000, but such cases are rare. In general it may be said that in cancer there is less anemia than the cachexia present would suggest, while in pernicious anemia the reverse is true. It is often said that a count below 1,500,000 is against cancer, but this rule often fails. In cancer one may expect to find fewer nucleated reds than in primary anemia and those present are more apt to be normoblasts, while a leucocytosis is more common. The highest of our series was 52,800. The leucocytes vary much in cancer of the stomach. A leucocytosis is present in over one-third of the cases and in those with normal counts a digestive leucocytosis is often absent (in 82% of 144 cases). Counts below 4000 are not rare.

It is said that the rapidity of growth of a gastric cancer influences the leucocyte count and yet our lowest counts included those with metastases in liver, pancreas, or peritoneum (1600, 5400, 5000, 5600), while in 15 cases of general carcinomatosis the leucocytes were above 10,000 in but 7. In one case the count was 105,000 (t.° 103° F.); in another, 24,500 (t.° 99°) and in a third, just before death, 61,400. These high counts were met with nearly always in cases with a slight fever.

Cabot reports a leucocytosis of 105,600 in a case with perforation into the peritoneum followed by quickly fatal peritonitis. We suspected this condition in a case the count of which rose to 120,000, an almost pure leucocytosis, but were unable to get an autopsy.

The percentage of large mononuclears is often high (1 to 10%) while in one case before death it was 33% of a total count of 6300 leucocytes (Kurpjeveit).

In Carcinoma of the Esophagus the blood is apt to be concentrated. This raises the percentage of dried substances, as in v.Noorden's cases, to 26.5 and 27.3%. And even in these cases also there may be an oligemia. If the cancer extends to the larynx, causing dyspnea, a high count may be due to cyanosis.

Of 6 cases, the highest red cell-count was 5,960,000 and the lowest 4,184,000. In another case on first blood examination the red cell-count was 4,696,000, hemoglobin 85% and 6000 leucocytes. A later examination in this case gave 6,476,000, 104% and 19,000 respectively. Five of the cases showed a leucocytosis. The highest count was 30,250.

In 15 cases of *general carcinosis of the abdominal organs,* cases in which one might assume a rapid and extensive growth, in but 2 was the red count below 4,000,000. In 27 cases of cancer of the bile-ducts the lowest red cell-count was 3,700,000 and in 5 of these the leucocyte count was above 10,000. (In 1 case it was 44,150; t° 100° F.)

In 4 cases of *cancer of the rectum* the lowest red cell-count was 3,732,000. The rest were about normal. There was a leucocytosis in 2 cases (13,100 and 19,750; t° 100° F.).

Of 10 cases of *cancer of the intestine* 3 showed a marked anemia. In 1, a cancer of the ileum, the red cell-count was 1,600,000, hemoglobin 40% and leucocytes 2500; in another the red cell-count was 1,780,000, hemoglobin 28% and leucocytes 10,000. This patient had nephritis also. In the third case, with cancer of the sigmoid flexure, the red cell-count was 1,609,000, hemoglobin 40% and leucocytes 7500. In 4 of the other cases the red cell-counts varied from 4,000,000 to 4,500,000. The highest count was 5,348,000. Of 9 of these cases in but 2 was a leucocytosis present.

Our other cases of carcinoma showed no striking features except 1 of the *testicle*, with 2,832,000 red cells and 9600 leucocytes. Cancers of the *kidney* are said to have usually high leucocyte counts, even 54,000, but we have seen no such case. In cancers of the *thyroid* the count may be 71,000 and in those of bone even 52,700.

Sarcoma has much the same effect on the blood as carcinoma and some think a worse. We can not believe this from the study of our cases unless the disease involves especially the bone-marrow or the lymph-glands. In those cases a severe anemia, resembling a primary pernicious anemia, and a high leucocytosis are the rule. In Hayem's case of osteosarcoma the red blood cell-count was 663,400; of v.Limbeck's 2 cases, in 1 the red cell-count was 1,118,000, hemoglobin 28% and leucocytes 68,200; in the other the red cell-count was 2,240,000, hemoglobin 48% and leucocytes 54,000. Yet other patients with this disease have counts even above 6,000,000. Nucleated reds are said to be less numerous in sarcoma than in carcinoma. The hemoglobin is said to be more reduced by sarcoma than by other neoplasms. The average given is about 50%. Thirty per cent. is a not rare figure and cases with hemoglobin even below 10% have been reported. The leucocyte counts in cases of osteosarcoma average about 17,000; that is, they are higher and the picture more often resembles leukemia than in cases of carcinoma invading the bone. The polymorphonuclear neutrophiles are less increased than by carcinoma but they may be increased when there is no leucocytosis. In some cases with little other evidence of metastases to bone the eosinophile cells are greatly increased, even to 50%. Myelocytes are sometimes present. The old question whether some of these white cells in the blood may not be free sarcoma cells is often raised, for it is the small mononuclears which are particularly increased.

Lues.—Lues, according to v.Limbeck, illustrates best the dictum that no one blood picture can be considered characteristic of any one disease. The blood picture in lues may simulate all other blood pictures from chlorosis to pernicious anemia of even the severest grade with a count of only 428,000 cells. Some cases of acquired lues have a practically normal blood, but this is unusual.

It is important not to confuse the anemia due to the disease itself, seen in untreated cases, with that due to vigorous mercurial treatment.

During the *primary stage* a severe chlorotic anemia is the rule. One following the large European skin clinics is struck by the weight given to this anemia, particularly in the case of women, in the diagnosis of a primary sore. Some say that at first, while the count remains normal, the hemoglobin will diminish considerably. Later, one of the first signs of the dissemination of the disease is the appearance of the skin rash and a further diminution of hemoglobin. The count may remain nearly normal notwithstanding a loss of hemoglobin of 25 to 30%. If the lues is untreated the hemoglobin may soon reach as low as 25% and the red blood-cell-count drop even at the rate of 23,000 cells per day. The severity of the anemia depends on the condition of the patient, his age, treatment, etc. In well-treated cases the regeneration of the blood is rapid.

Leucocytes.—In an adult a high lymphocytosis and an eosinophilia would suggest lues. In a child this blood picture might suggest rickets also. A low hemoglobin per cent. and a high percentage of small mononuclears would indicate a severe case.

The leucocytes in the primary stage are normal, or there is a slight leucocytosis with an increased percentage of lymphocytes. If mercury is given the percentage of the polymorphonuclear neutrophiles will rise. This is the reverse of the action of mercury in a normal case.

During the secondary stage the leucocytes vary from 12,000 to 16,000, due to an increase of lymphocytes and eosinophiles. The latter are increased especially with the papular syphilide.

The *tertiary stage* is often accompanied by a severe anemia and a leucocytosis due to a high lymphocytosis, which is of aid in excluding pernicious anemia. Myelocytes are present in severe cases.

In 19 cases of secondary lues the red cells were but slightly diminished. (The minimum count was 4,200,000. In 6 it was above 5,000,000.) The hemoglobin was more affected than the count of red cells. (This varied from 40 to 90%, the mean, 75%; and the color-index varied from 0.5 to 0.9, the mean 0.7.) The leucocytes, as a rule, were normal (in 11 cases below 10,000, in 3 between 10,000 and 12,000) except in 5 cases with high fever (luetic fever of secondary stage) in 4 of which they ranged between 12.000 and 24,000 and dropped with the temperature.

There was a slight rise of the leucocyte count during the primary stage (the average was 9000). During the secondaries the count, depending on the skin lesion and on the fever, varied from 9000 to 24,000 (in 1 case even 50,000), the average from 12,000 to 15,000. During the tertiary stage the counts varied greatly; in some cases there was a slight rise, in others a leucopenia. In hereditary lues the count has been found high, from 12,000 to 24,000.

In hereditary and tertiary lues the red cells are seriously affected in number, size and color. Megaloblasts are common. The blood picture, especially of the long-standing cases, may resemble that of primary pernicious anemia; yet, as in cancer, the megalocytes do not predominate as they do in pernicious anemia. Many cases of anemia in children reported as luetic were probably cases of anemia pseudoleukemica infantum. Miller reported a case with 720,000 reds and 18% hemoglobin, with normoblasts, megaloblasts, even gigantoblasts, microcytes and poikilocytes present. The anemia of the hereditary lues of infancy may be fatal. The average leucocyte count of 25 cases was 7050. Large nucleated reds containing little hemoglobin aid in diagnosis (Cima).

Following mild mercurial treatment the red cells may rise even 100,000 cells a day for about 14 days, until even a slight hypercythemia is present. But this rise is often preceded by a drop (Justus' test, see below) but sometimes with a hemoglobinuria which is followed by rapid regeneration. If the mercurial treatment is carried too far, that is for 24 or more days, it may itself produce an anemia.

Of 23 of our cases, 7 of which were of cerebral lues, the red cells were above 5,000,000 in 9 cases and between 4,000,000 and 5,000,000 in 10. The lowest count was 2,870,000. The color-index varied from 0.4 to 0.9, the mean was 0.67. The leucocytes were below 10,000 in 20 of 29 cases. In the other 9 cases they varied from 10,000 to 18,500 (this case had large gummata); 6 were cases of high luetic fever; another was of the malignant type (leucocytes 16,000, no fever). In 1 cerebral case the leucocytes numbered 3000, in another 2100.

Justus' Test.—If a large inunction or injection of mercury is administered a patient with lues before the rash and yet after there is general glandular enlargement, and also during the secondary or tertiary stages, and in hereditary lues provided the disease is at the time advancing, the hemoglobin will at once drop from 10 to 20% and during the next few days will return to normal or even to above normal with improvement of all the symptoms. This drop, which is both rapid and considerable, is said to be specific for a case of active lues. It is not positive during the primary stage while the infection is limited to the chancre and its neighboring glands, therefore it cannot be used early to differentiate between a hard and a soft chancre.

The explanation suggested is that the mercury destroys the red blood-cells which are already damaged by the disease and stimulates the production of new ones.

This test is not as specific as was first claimed and yet it is valuable.

Renal Disease.—The kidneys play an important part in the control of the composition of the blood, hence in nephritis the plasma changes are early and important: a loss of albumin, a lowered specific gravity and in general all the signs of a chronic secondary anemia. For the further chemistry of the blood in nephritis see pages 539 and on.

IN ACUTE HEMORRHAGIC NEPHRITIS especially the count may fall to a low point, even to 1,000,000, but usually the anemia is moderate, and of this much is only apparent.

In 12 recent cases of *acute nephritis* there were but 2 low counts, 2,600,000 and 2,900,000; there was a leucocytosis in 5 of from 11,400 to 18,900. Of Cabot's 50 cases, the lowest red count was 3,568,000 but the leucocytes were above normal in 31 of these cases. (The highest was 50,000.) Cabot thinks the leucocytosis due in part to hematuria or uremia. But since nephritis is, or is part of, an acute febrile and probably infectious disease a leucocytosis is to be expected.

In *chronic nephritis* many factors come into play, nearly all of which tend to produce an anemia. Among them are a subacute often latent pyogenic infection; the disturbances of circulation; the edema and hydremia; the disturbances of the gastro-intestinal tract, vomiting, diarrhea, poor appetite, and the influence of the purges. The result is often a lowered count, a still more lowered hemoglobin per cent. and a hydremic plasma.

In 103 cases of chronic nephritis the red cells were 1,700,000 in 1 case, between 2,000,000 and 3,000,000 in 13 cases, between 3,000,000 and 4,000,000 in 25 and over 5,000,000 in 19. The mean was 4,500,000.

The hemoglobin in 99 cases was between 20 and 30% in 3 cases, from 30 to 50% in 29 and above 80% in 17. The mean was 62%. The mean color-index was therefore 0.7, which is about normal.

The leucocytes in 80 cases without uremia were below 5000 in 4 cases, from 5000 to 10,000 in 43 and above 10,000 in 33. (The highest were between 20,000 and 30,000.)

In 33 cases with uremia the highest leucocyte count was 25,900. It was above 10,000 in 15 cases and below 5000 in 2. The mean was about 9000. It is seen that in our cases the uremic syndrome was not mirrored in the blood.

The cases of nephritis which resemble pernicious anemia form a most interesting group.

The case which Dr. McCrae[154] reported is a good illustration of this. The patient was a man 39 years old whose red cell-count was 1,400,000, hemoglobin 27% and leucocytes, 7000 (pmn. n. 88%; s.m. 8%; l.m. 2%; and eos. 2%). There was no poikilocytosis and but 1 nucleated red was found. The urine was of low specific gravity and contained much albumin and many casts.

Cabot reports such a case with 1,468,000 reds, hemoglobin 23% and leucocytes 3800 (pmn. n. 70%; l.m. 4.4%; eos. 2.6%; megaloblasts, normoblasts and poikilocytes were present).

In the case of Labbé the red blood-count was 500,000 and the hemoglobin 2 gms. The cells were pale and irregular in form and size. The nucleated reds were rather small. Mononuclears made up 50% of the leucocytes. Recovery was rapid. He suggests that anemia was for the most part apparent and due to the dilution of the plasma. In another case the red blood cell-count was 418,500 and the color-index over 1; and in a third the count was 1,000,000. At autopsy in such cases nephritis was the only lesion found. There were in these cases practically no signs of blood destruction, nor of regeneration, nor of megaloblastic degeneration of the marrow.

We mention 2 other cases of chronic nephritis with marked arteriosclerosis. One was a woman 54 years of age, with red cell-count 2,800,000, hemoglobin 50%, and leucocytes 6000. The other was a man 32 years old, with red cell-count 1,772,000, hemoglobin 22% and leucocytes 50,000 (of which 91% were pmn. n.). The leucocytes later rose to 116,000. He left the hospital unimproved.

In *interstitial nephritis* the count is normal at first, and sometimes remains so to the very end. The condition of the heart is in this connection important. During the acute exacerbations of the nephritis, however, a slight lowering of the count is common. This is perhaps due to the hydremia. In 2 cases of *bilateral cystic kidney* the red cell-counts were 1,200,000 and 2,800,000; and the leucocyte counts, 13,500 and 36,000.

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[154] Johns Hopkins Hosp. Bull., October, 1902, p. 245.

Diseases of the Liver.—CATARRHAL JAUNDICE.—"Occasionally in catarrhal jaundice there is a slight leucocytosis at the onset but otherwise the blood is normal, although some degenerative changes of the red cells are met with in severe cases" (Cabot). An increase in resistance, in rigidity and in size is claimed for the erythrocytes.

Of 27 of our cases the red count was normal or even above normal in 16; the lowest was 3,000,000; and the mean, in the male patients, 5,000,000. An interesting feature was a rise in this count while in the hospital of from 300,000 to 750,000 cells. Of the 27 cases, in 20 the leucocyte count was 10,000 or below; in 3, from 10,200 to 19,500; and in 4 from 14,200 to 19,500. These cases all had a slight fever. These cells fell rapidly to normal after admission. A leucopenia may follow in some cases (Bezançon and Labbé).

The plasma is bile-stained. The coagulation time slow.

TOXIC JAUNDICE.—There were in the Johns Hopkins Hospital clinic 3 fatal cases of toxic jaundice. In 1 the red cell-count was 3,570,000, hemoglobin 65% and leucocytes 11,400. In the second, these figures were 5,280,000, 75% and 7000; and in the third, 5,400,000, 65% and 12,500 respectively.

GALL-STONES.—A mild leucocytosis is the rule during an attack of gall-stone colic; a high one is rare. In the Johns Hopkins Hospital's 36 cases these cells rose suddenly during the colic to about 15,000, but in the cases of stone in the common duct with chills and fever, they rose even to 24,700. The red cell-count varied from 2,800,000 to 6,400,000, the mean was 4,300,000. In a case with hemorrhage they fell to 1,880,000, the hemoglobin to 23%, while the leucocyte count was 17,500. The coagulation time of the blood of a jaundiced patient is often lengthened and so should be tested before any surgical operation which should be postponed until, as the result of therapy, this becomes normal.

CHOLECYSTITIS.—In cholecystitis the leucocyte count is invariably high, from 20,000 to 27,000 (Bloodgood). In 1 of our cases it was 46,500. As the case becomes chronic the count falls nearly or quite to normal.

CHOLANGITIS.—Of 5 cases of cholangitis the leucocytes were 16,000, 33,160 (fatal), 15,600 (t.° 103.5°), 9000 (t.° 103°) and 6,400 (t.° 106°; fatal).

ABSCESS OF LIVER.—In cases of abscess of the liver the leucocyte counts are high while the temperature is high, but are lower or normal when the temperature is normal. Futcher[155] found the average in 15 cases to be 18,350 and the maximum 53,000. The red cells in these cases varied from 2,600,000 to 5,600,000, the mean, 4,200,000; the mean of hemoglobin was 60%.

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[155] Jour. Am. Med. Assoc., August 22, 1903.

Cirrhosis of the Liver (Atrophic).—Early in cases of cirrhosis of the liver the red cell-count is normal while later an anemia may develop. Da Costa's average was 3,404,000 and Cabot's, 3,580,000. In 1 case it fell to 1,300,000. The leucocyte counts are normal or low.

In the Johns Hopkins' 32 cases the red cells varied from 3,100,000 to 5,900,000, the mean 4,500,000. The hemoglobin mean was 68%. The leucocyte counts in 30% of our cases were over 10,000 and the highest was 16,000.

Hypertrophic (Hanot's) Cirrhosis.—Hayem reported a case with extreme anemia.

In the Johns Hopkins' series there were 5 cases. In 2 the count was high, 7,800,000 and 8,500,000, and in 1 as low as Hayem's case— 1,504,000. In this last case the hemoglobin was 28% and the leucocyte count 6100. (This count rose later.) In 2 there was a leucocytosis (11,000 and 12,800).

Acute Yellow Atrophy.—In the cases of acute yellow atrophy thus far reported the red cell-counts were normal and the leucocyte counts slightly elevated. In one case of the Johns Hopkins' clinic, a boy 14 years old, the red count was 4,800,000 and the leucocyte count 12,700.

Leprosy (v. Limbeck).—In leprosy the blood may for years be normal but later a pseudochlorosis develops with a normal leucocyte count. After general malnutrition begins the anemia becomes more marked and yet is rarely very severe. In 1 case, however, the red cell-count was 2,290,000 and the hemoglobin 55%. The leucocyte counts have varied from 4000 to 8000 per c.mm.

Heart Disease.—While cardiac compensation is good the blood is normal, but with acute dilatation and fall of the blood-pressure the blood becomes hydremic, hence the count falls. Later with chronic passive congestion and cyanosis the red cell-count rises and so may conceal an anemia. The most marked anemia is seen in aortic valvular insufficiency, as in 1 case with red cell-count 3,400,000, hemoglobin 30% and leucocytes 8000. If, as the result of proper therapy, the anemia improves this may aid the heart to regain its compensation. In congenital heart disease with extreme cyanosis the picture is particularly interesting since there often is a polycythemia, the red cell-count between 8,000,000 and 9,000,000.

During the loss of compensation in 29 males with pure mitral disease the count varied from 3,000,000 to 7,500,000, the mean 6,200,000. In 46 women the mean was 4,700,000, but the extremes were 3,500,000 and 8,000,000. One noted interesting jumps of from 1,000,000 to 2,000,000 cells while these cases were under treatment.

In 37 cases of pure aortic disease the mean red cell-count was 5,200,000. These cases as a rule showed a lower count on each successive admission.

In 29 cases of arteriosclerosis (no important cardiac lesions) the mean was 5,200,000. In 34 cases of aneurism of the thoracic aorta the mean was 5,500,000 and in 5 men with aneurism of the abdominal aorta it was 4,500,000

Addison's Disease.—A hypocythemia is the rule in Addison's disease, the red cell-counts varying from 2,000,000 to 3,000,000. In 1 case it was 1,120,000. In other cases, however, the count may be even above 7,000,000. Some consider that any anemia in this disease is due to a complication; others, that there is always an anemia but that this is covered by a concentration of the blood, and cite a case with true oligemia and a count of 4,774,000 cells.

Myxedema.—In myxedema the red cell-count may be normal, above normal, or diminished. Many report an anemia which improves with treatment. Some report that the diameter of the red blood-cells is increased and also the presence of many nucleated reds (that is, an infantile condition of the blood). The platelets in a recent case were much increased.

Rickets.—Anemia, generally of a mild grade, is the rule in rickets, but sometimes it becomes severe, rapid in its development and even pernicious.

Scurvy.—The red cell-counts reported in scurvy have varied from about 3,000,000 to 4,000,000 cells. The cases with many hemorrhages have a much more intense anemia. In Buchard's case, *e. g.*, after 3 weeks with considerable epistaxis, the count was 557,000. In some grave cases macrocytes, microcytes and fragmented reds have been found. The color-index is reported low.

The Blood in Inanition.—The effects on the blood of a healthy man of a 31-day fast were studied by Ash[156] who found a very slight actual loss in hemoglobin, more marked during second 10 days (of but about 7%), moderate fluctuations in the water content of the blood, particularly during first half of the period, a decided rise in polymorphoneutrophiles in the early days (to 79%) and an increase in coagulability, especially after the first 2 weeks (coagulation time on the 21st day was 55 seconds). He found the blood as a whole distinctly resistant to the effects of uncomplicated inanition.

THE VALUE OF BLOOD EXAMINATION

By the examination of the fresh blood the diagnosis of some cases is made or is suspected. Among these are malaria, especially the forms without definite paroxysms, which often pass as typhoid fever, meningitis, uremic coma, pernicious anemia, appendicitis, tuberculosis, dysentery and even Raynaud's disease (cases with superficial gangrene). The failure to look at a smear of blood may in these cases result in the unnecessary death of a patient. And yet not every patient with malarial organisms in his blood should then be treated for malaria. He may be a chronic carrier who now has typhoid fever, etc. Trypanosomiasis can be recognized by the blood and the Leishman-Donovan bodies by splenic puncture. Pernicious anemia is often overlooked although a cursory glance at a fresh blood specimen would save some patients from a course of treatment for jaundice, peripheral neuritis, or tabes. The practical value of 3 minutes spent making and glancing at a fresh blood specimen is shown by the fact that the majority of our cases of myelogenous leukemia come to the surgical side for "abdominal tumor." The diagnosis of lymphatic leukemia, acute leukemia and pseudo leukemia can be made only in this way.

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[156] Arch. of Int. Med., July, 1914, xiv, p. 8.

For the early diagnosis of typhoid fever, measles and influenza a leucopenia is valuable, while a leucocytosis would speak in favor of pneumonia, scarlet fever, acute epidemic cerebrospinal meningitis and various abscess formations, as of the liver or brain, etc. Early in typhoid fever a blood culture often settles the question, later a Widal test might do so.

The presence of a leucocytosis is very valuable in the diagnosis of pneumonia, especially central, and that of children and drunkards. In an ever-increasing number of cases of trichinosis the diagnosis has been suggested by the eosinophilia alone while the blood examination has the same value in cases of chronic poisoning with coal-tar products notwithstanding the denials of the patients. Various tuberculous infections are thus differentiated, also the secondary anemias due to cancer from primary anemia, etc.

For the surgeon, blood examination is usually synonymous with leucocyte counting. The question he usually asks is, "Should I operate or not?" on a doubtful case of appendicitis, typhoid perforation, etc. In these cases the leucocyte curve should be determined rather than a single count.

Both the medical man and the surgeon should remember that 1 count is seldom enough, any more than is 1 temperature reading. It is the curve that has value.

For American students the message is, less routine blood-work but a better quality of that which is done. The examination of the fresh specimen will save a great many unnecessary routine counts. Also interpret your findings in terms of the individual case, for changes in blood cells or serum are symptoms to be translated, not signs to be obeyed.

MALARIA

The following are a few of the terms used in this chapter which may need definition. *Schizogone*, the asexual generation; *schizont*, or *monont*, an individual organism belonging to the asexual generation; *merozoite*, a segment, *i.e.*, a hyaline; *gametoschizont*, the sexual generation; *gamete form*, 1 organism of the sexual generation. Of the gamete forms, the *macrogamete* is the female cell; the *microgametocyte*, the parent male cell, and the *microgamete* is the male cell, *i.e.*, is 1 " flagellum " of the microgametocyte. *Sporogone*, the cycle in the mosquito; *vermiculus* or *oökinet*, the motile fertilized macrogamete; *zygote*, *oöcyst*, *sporoblast*, are terms given to the spore cysts; *sporozoit*, the young sexual form which develops in the sporoblast and which, when inoculated into the human blood, becomes a hyaline.

By *malarial pigment* is always meant the transformed hemoglobin, the brown granules of which are seen in the fresh specimen. This term is never used of chromatin granules.

Hyaline always means a non-pigmented young form. A *ring form* is the shape which any hyaline may assume; it is not a "kind" of organism. *Presegmenters* are full-grown parasites before segmentation has appeared, the pigment of which has accumulated into 1 or a few masses. The term *granule* is used in several senses when describing malarial blood. It may mean a particle of malarial pigment, or a small mass of chromatin, or a degenerated area of the red blood-cell (Plehn's granules).

The examination of the fresh malarial blood is often more satisfactory than is that of the stained specimens since for the diagnosis of the various forms of parasite the color of the red cell, the refractivity of the parasite's protoplasm, the rapidity of its ameboid motion and the speed of vibration of its pigment, all are important. It also is true that one is less likely to be deceived by artifacts in fresh than in stained blood. On the other hand the parasites are easily found in stained specimens and, when very few, the Ross method alone may make diagnosis possible.

The Organism of Tertian Fever; Hemameba Vivax (Grassi); **Plasmodium Vivax** (Plate III).—This is the commonest form of malaria in this country. Since the cycle of development of this parasite requires approximately 48 hours the paroxysms in the case of a single infection will occur on alternate days, granting that the infection is intense enough to cause paroxysms. In case there is a double infection there will be a paroxysm each day, "quotidian" fever, and the 2 groups will be seen in the blood. Three groups very rarely occur, but we have seen 2 or 3 cases. The grouping of these organisms is fairly definite, *i. e.*, all of the parasites of 1 group develop quite in unison. The paroxysms occur during segmentation and last from 12 to 14 hours. The hyaline form of the tertian parasite (2-4) is a little over 2μ in diameter, is colorless, non-pigmented and often disk-shaped, with an undulating periphery. It is either on or in a cell which even now may be a trifle swollen. It now makes very rapid ameboid movements, which produce an extraordinary series of changes of shape and position and which at any time may assume the typical ring form once supposed to be characteristic of the æstivo-autumnal parasite. This ring is usually a little thicker at one point, due to the mass of chromatin, hence the name "signet ring form." In one red cell may be 1, 2 or even 5 such hyaline forms. In about 12 hours the infected corpuscle (6-7) will be a little larger, a little paler, but still has a sharp, smooth, round margin. The organism is exceedingly ameboid. The pseudopods often are numerous and so thread-like and pale that their connections can scarcely be seen and so the cell seems to contain a number of disconnected globules of pigmented protoplasm. The protoplasm is so little refractive that the outline of the parasite is difficult to make out. The malarial pigment is now present. It is moderate in amount, massed at the ends of the pseudopods and consists of very fine, light-brown granules, which dance with a motion so rapid that waves in the protoplasm must be assumed to explain it. At

PLATE III

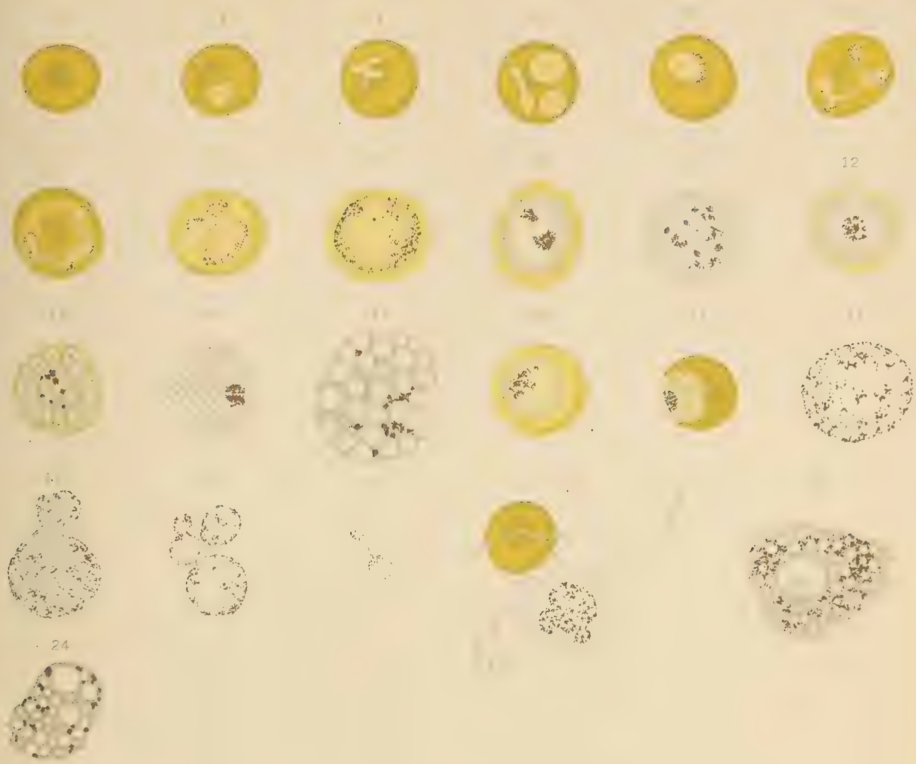
The Parasite of Tertian Fever. (Drawn by Mr. Brödel for Thayer and Hewetson's paper, The Malarial Fevers of Baltimore, Johns Hopkins Hospital Reports, Volume V.)

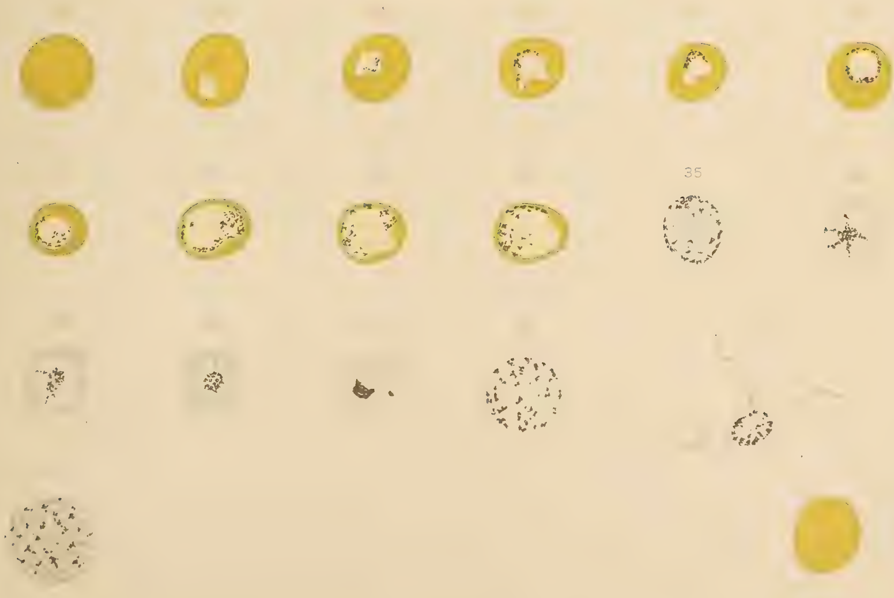
1. Normal red corpuscle.
2, 3, 4. Young hyaline forms. In 4, a corpuscle contains three distinct parasites.
5, 21. Beginning of pigmentation. The parasite was observed to form a true ring by the confluence of two pseudopodia. During observation the body burst from the corpuscle, which became decolorized and disappeared from view. The parasite became, almost immediately, deformed and motionless, as shown in Fig. 21.
6, 7, 8. Partly developed pigmented forms.
9. Full grown body.
10–14. Segmenting bodies.
15. Form simulating a segmenting body. The significance of these forms, several of which have been observed, is not clear to the writers, who have never met with similar bodies in stained specimens so as to be able to study the structure of the individual segments.
16, 17. Precocious segmentation.
18. Macrogamete.
19, 20. Fragmenting extra-cellular bodies.
22. Microgametocyte.
23, 24. Vacuolization.

The Parasite of Quartan Fever.

25. Normal red corpuscle.
26. Young hyaline form.
27–34. Gradual development of the intra-corpuscular bodies.
35. Full grown body. The substance of the red corpuscle is no more visible in the fresh specimen.
36–39. Segmenting bodies.
40. Female gamete.
41. Microgametocyte.
42. Vacuolization.

PLATE III.





the end of 24 hours the cell (Plate III, 8) is somewhat larger and paler but still round in outline. The organism now fills about ⅓ of the cell. That is, it has lived ½ its cycle and yet has attained but ⅓ of its adult size. It is still ameboid, but less actively so. The pigment has increased in amount, is a little darker, a little coarser, a little quieter and is now evenly distributed through the substance of the parasite. The nucleus of these forms sometimes can be seen in the fresh specimen as a globular body at the end of a pseudopod.

During the latter half of the cycle the growth of the parasite is much more rapid. At 40 hours the parasite (9) is practically full-grown. The red cell is now about 1½ times its normal size and so pale that its outline will hardly be seen. The organism is from 8 to 10μ in diameter, is round and so little refractive that it is practically impossible to distinguish between parasite and corpuscle. The pigment is more abundant and is evenly distributed throughout the parasite, an important point in diagnosis since in the quartan at this age it will be practically all in the periphery, and in the æstivo-autumnal at the center.

The next stage is the "presegmenter." The corpuscle is now almost or quite invisible. The pigment granules move in regular lines to collect in 1 or more irregular clumps. The organism is now a "segmenter" (10-17). It becomes slightly more opaque, more refractive, and the corpuscle is no longer seen. Refractile dots, from 15 to 20 in number, appear irregularly in the body of the protoplasm, crenations are seen at the margin and lines of separation appear around these refractive dots marking off the future segments. The segments now become more sharply defined until finally the parasite is a clump of 15 or 20 discrete circular masses with a refractive dot in the center of each, irregularly arranged or forming 2 quite concentric circles. The pigment is left in masses between these segments. The segmenter now seems to burst since the young organisms suddenly spring apart. Each segment is a hyaline ready for a new cell as host.

The whole cycle may occur in the peripheral blood, but the number of segmenters found will not be as large as would be expected from the number of parasites seen a few hours previously, since so many of them have accumulated in the internal organs. A few hours after the first segmenters appear the chill begins.

The above is a description of a typical tertian parasite. One finds, however, forms which differ somewhat. In one, the parasite develops more pigment, in large coarse granules of a lighter brown color than that of the quartan or the adult æstivo-autumnal and these form dense clusters at the ends of the pseudopods filling them so completely that the granules cannot dance at all. The fine thread-like pseudopods of these parasites stand out with great distinctness. The infected cell is often not much swollen but is very pale. In one such case, however, all the full-grown forms found were in cells from 8.5 to 13.3 μ in diameter. Pigmented leucocytes are common (perhaps since the pigment granules are so conspicuous).

The grouping of this form does not seem to be as definite as that of the typical tertian and so the chills are slightly longer than usual.

EXTRACELLULAR TERTIAN FORMS.—It is not at all uncommon for the tertian parasites to burst from their cells and die (19-21). These degenerated forms may, a short time after the specimen is made, be the only ones seen. The organism is often seen to "run out" of the red cells as if through a very fine hole, leaving but a shadow of the cell. After the parasite is free in the plasma the pigment gradually becomes quiet, the protoplasm may then break up into fragments, forming a string of 4 or 5 small pigmented spherical masses (20-21), or it may become deformed, swollen and vacuolated, the formerly so-called "sporulating forms" (23, 24).

The tertian gametocytes would appear in fresh specimens to be full grown forms, often extracellulars, but when stained the shell of the corpuscle may be seen. Like the crescents of the æstivo-autumnal parasite to which these correspond, they can be found in the blood at all times after the infection has continued for a few days. The macrogamete (18) formerly was considered a cadaveric form, and was known as a "swollen extracellular." These large organisms, some 3 or 4 times the size of a red blood cell, have abundant pigment often in the form of coarse rods which are in very active movement. Their nucleus is about 3.5μ in diameter and is often evident in the fresh specimen as the only portion of the parasite which the pigment granules do not invade. The extreme vitality of these cells is astonishing (as might be expected from the fact that it is their function to continue the life of the organism in the mosquito) for the pigment will dance actively for even after 18 hours if the microscope is kept in a warm room. The microgametocytes are from 8 to 10μ in diameter. When studied on the warm stage their pigment, at first in active motion, soon forms a circle around the center. Then, as though stirred up by something within the cell, the margin may undulate and the "flagella" (erroneously so called), 4 or 5 in number, burst out. These "flagella" are the microgametes or male elements. They are threads of chromatin from 2 to 3 times the diameter of a red blood-cell in length and often rendered irregular by fusiform masses of protoplasm containing pigment granules. These microgametes break loose and thrash their way for more than an hour among the red cells leaving behind only a small cell with its pigment near the center. This "flagellation" is seen first in from 15 to 20 minutes after the blood has been drawn which is proof that it does not occur in the body, but under the stimulus probably of the lowered temperature.

Hemameba Malariæ, the Parasite of Quartan Malaria (Plate IV).—Of this rare form we see few cases now although in some localities it is at times the predominant organism. Since its cycle of development requires 72 hours, if but one group is present there will be one paroxysm on each fourth day; if 2 groups, there will be 2 days with paroxysms and then a free day, followed by 2 more paroxysms, etc.; if 3, the patient will have quotidian fever, providing each group is large enough numerically to cause a chill. The grouping of this parasite is even more uniform than is that of the tertian; that is, the forms of the same group keep more nearly of the same age and hence the paroxysms are slightly shorter, often requiring but 10 hours.

The quartan hyalines (26) cannot be distinguished from the tertian parasite, but a little later, when the pigment appears (27), the granules are seen to be coarser, blacker and less actively vibratory than the tertian. As the parasite grows the infected cell becomes smaller, shrunken and its margin irregularly crenated, but much deformity is rare. The protoplasm of the parasite is very refractive so it can easily be seen. This parasite is only sluggishly ameboid.

In 24 hours the infected cell is still smaller, is crenated and brassy in color. The organism is very distinctly made out since so refractive, is oval and sluggishly ameboid. The pigment is coarse, blackish-brown in color, is clustered at the periphery, especially on one side, and is quiet. The parasite soon fills from one-third to one-half of the cell (30, 31), becomes rounder, and is no longer ameboid. The infected cell may be more shrunken, more crenated and more brassy, although some seem but little altered. The coarse, black pigment is entirely at the periphery.

During the third day only a rim of the red cell is left and this is usually of a dark, brassy color. The organism (32-34) is now full-grown and averages, most authorities say, about 7μ in diameter.

Of 135 full-grown quartan parasites, some of them segmenters, which we have measured, 60% were from 7.4 to 8.1μ in diameter, and only 18% from 6.2 to 7μ. Of those ⅔ grown, 43% were in cells from 6.2 to 7μ in diameter, the rest in cells of normal size.

At 60 hours the red cell is scarcely visible. The organism is round or elliptical and motionless (35). The coarse dark pigment is all at the periphery.

The pigment now flows to the center in definite streams along definite radial channels, thus giving a beautiful wheel-like picture (36), and finally collects in a clump at the center. These are the presegmenters. The segmenters present an attractive picture. The organism becomes very waxy in appearance; a single row of refractive dots form a circle at the periphery which becomes crenated around each and lines of division start from these crenations and run to the center, forming from 6 to 12 rays like the petals of a flower, hence the names "daisy," "Marguerite," or "rosette" form (37, 38). These segments then separate as in the tertian. The quartan lives its full cycle in the peripheral blood, hence the number of parasites in the peripheral blood does not diminish as the parasite grows older.

The gamete forms (40, 41) are seldom seen. They are similar to but somewhat smaller than those of the tertian. Flagellation occurs in the same way. The extracellular degenerated forms are found, although the parasite keeps within the cell much better than does the tertian.

Æstivo-autumnal Fever; Plasmodium Precox; Hematozoon Falciparum (Plate IV).—Æstivo-autumnal fever is a common form of malaria particularly in the Tropics and is the most dangerous of the three. In fresh infection the grouping of the organisms is quite definite and the fever definitely intermittent, but soon the members of a group lose their unison of growth and parasites of all ages may be found in the same blood (and spleen) as a result of which the fever becomes more and more continuous. The duration of the cycle is rather uncertain. Doctor Thayer considers that while usually it is about 48 hours, it may vary from 24 to perhaps 72.

All students, it is said, pass through the stage of desiring to divide this form into "benign," a "malignant," or "pigmented," "non-pigmented," etc., varieties, but most recover, especially those who follow the splenic blood carefully. In this locality we are often impressed by the great differences seen in the æstivo-autumnal parasite, especially as regards the amount of pigment it develops and the number of adult forms which appear in the circulation; but no division of this group has stood the test of time.

The hyalines of the æstivo-autumnal are similar to those of the tertian and the quartan except that they are perhaps slightly smaller and are more apt to assume the signet-ring form. They are very refractive, hence easily seen, but may at any time lose this refractivity and become ameboid exactly as does the tertian. In a severe infection even five rings may occupy one cell.

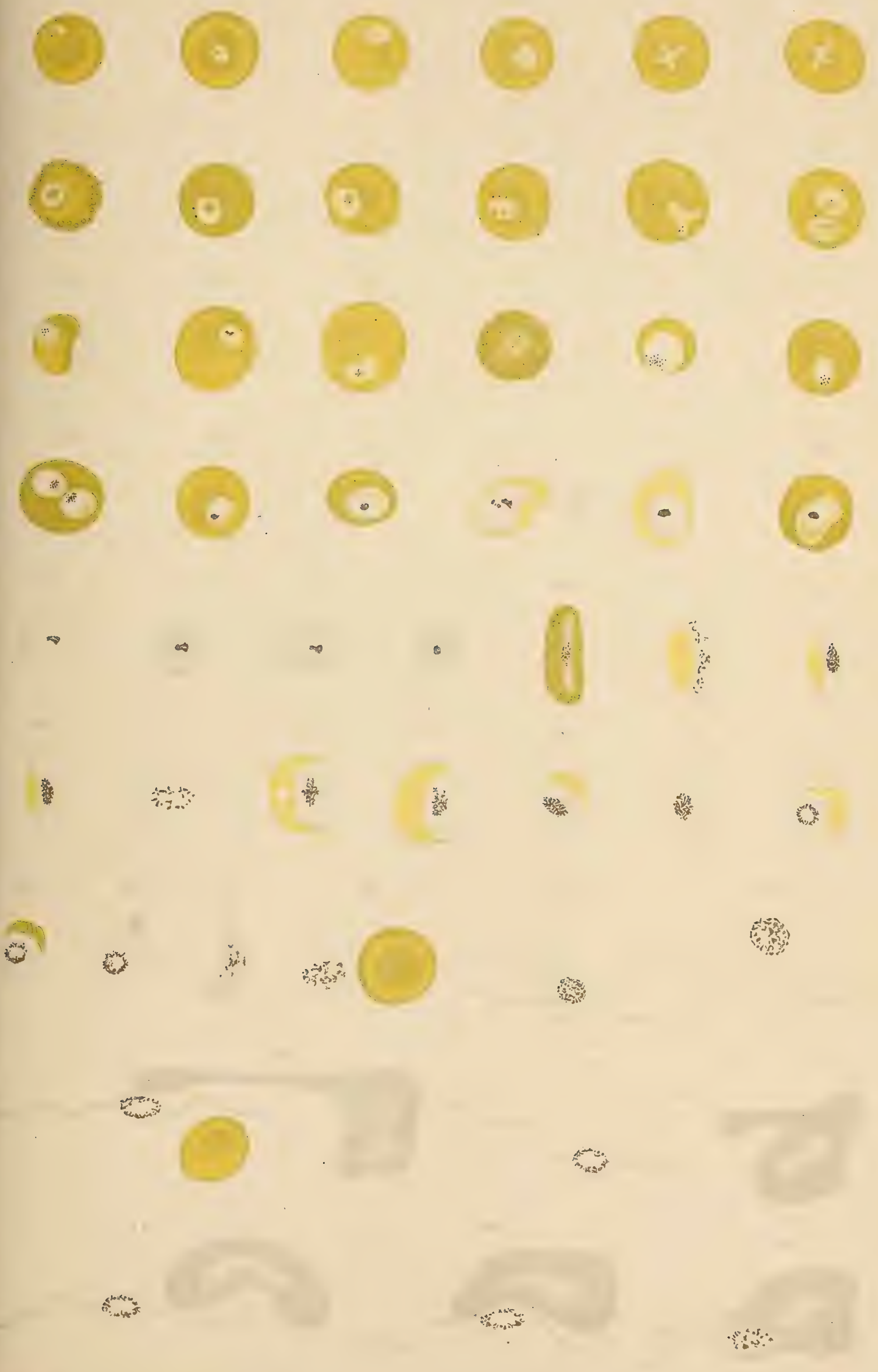
As the parasite (7-12) grows a very slight amount, usually but 1 or 2 granules, of pigment appears. These are so fine that they are easily overlooked, are motionless as a rule, although sometimes they dance slightly and for the most part are seen at its periphery. The infected red cell is usually much shrunken, crenated and brassy, even when the parasite is young, and yet some infected cells appear normal. Many red cells which contain no parasite also show similar changes. The parasite at this stage fills about ⅕ of the cell. As a rule the infected cells now disappear from the peripheral circulation and to study their further development the spleen must be punctured. Their departure is so sudden that in 2 hours a large brood may disappear. Rarely are older parasites found in the peripheral blood (Fig. 117). In these cases and in the blood obtained by splenic puncture, the pigment increases considerably in amount as coarse, dark granules, although some produce practically none. The former may easily be mistaken for quartan forms. The more malignant the parasite the fewer older forms are seen in the peripheral blood (although in pernicious cases there is always an abundance of young parasites in the peripheral blood) and, according to

PLATE IV

The Parasite of Æstivo-Autumnal Fever. (Drawn by Mr. Brödel for Thayer and Hewetson's paper, The Malarial Fevers of Baltimore, Johns Hopkins Hospital Reports, Vol. V.)

1, 2. Small refractive ring-like bodies.
3–6. Larger disc-like and ameboid forms.
7. Ring-like body with a few pigment granules in a brassy, shrunken corpuscle.
8, 9, 10, 12. Similar pigmented bodies.
11. Ameboid body with pigment.
13. Body with a central clump of pigment in a corpuscle, showing a retraction of the hæmoglobin-containing substance about the parasite.
14–20. Larger bodies with central pigment clumps or blocks.
21–24. Segmenting bodies from the spleen. Figs. 24–27 represent one body where the entire process of segmentation was observed. The segments, eighteen, in number were accurately counted before separation as in Fig. 28. The sudden separation of the segments, occurring as though some retaining membrane were ruptured, was observed.
29–37. Crescents and ovoid bodies. Figs. 34 and 35 represent one body which was seen to extrude slowly and, later, to withdraw two rounded protrusions.
38, 39, 42. Round bodies.
40. "Gemmation," fragmentation.
41. Vacuolization of a crescent.
43–44. Flagellation. The figures represent one organism. The blood was taken from the ear at 4.15 P.M.; at 4.17 the body was as represented in Fig. 43. At 4.27 the flagella appeared; at 4.33 two of the flagella has already broken away from the mother body
45–49. Phagocytosis. Traced by Dr. Oppenheimer with the camera lucida.

PLATE IV



some, the less pigment is formed. In some cases the hemoglobin seems to gather around the parasite leaving an almost colorless ring of the infected cell at the periphery (13). In the internal organs the cycle seems to develop inside of large macrophages. The full-grown parasite is very characteristic in appearance. It is about ½ the size of the cell (5μ), its protoplasm is waxy and its pigment is all in the center (15-20), never diffusely scattered and never at the periphery. The segmenters vary in size from 2.5 to 5μ in diameter. The process of segmentation (21-24) resembles that of the tertian in that the organism breaks up irregularly into 15 or 16 very small segments. Very few degenerated extracellulars are found.

Crescents and Ovoids.—The gamete forms of the æstivo-autumnal parasite have the same significance as those of tertian and quartan malaria but differ in that they have the perfectly characteristic and easily recognizable shapes of crescents and ovoids. These are found in the internal organs after about the fifth day of a fresh infection and appear in the peripheral blood on about the seventh day. The crescents (29) are slightly longer than the diameter of the red blood-cells, are very refractive, have a double contour and usually are surrounded by a fringe of the shrunken red cell which in the concavity forms the so-called "bib." The pigment, in coarse and usually rod-shaped granules, is considerable in amount and is clustered at the center of the crescent either as a confused mass, a sheaf, or a ring. While watching the gamete it may lose its crescentic shape and become, first an oval (ovoids, 30-33), then a circular form (34-36), or it may resume the crescentic shape. Around the circular no trace of the corpuscle is left and its protoplasm is much less refractive than that of the crescent. Two forms of the circulars have been described in the fresh blood, the macrogamete and the microgametocyte (see page 662). The latter may flagellate and fertilize a macrogamete.[157]

The phagocytes of the blood can be well studied in this form of malaria. In fact, the discovery of a pigmented leucocyte is almost as valuable as that of the parasite itself. These are the large mononuclears especially, also the polymorphonuclear neutrophiles and macrophages not usual in the peripheral blood. (See page 507 and Fig. 117). These phagocytes contain free pigment granules, or masses of pigment, or parasites, especially segmenters and flagellates. In the tertian and quartan they appear just after a chill, but in the æstivo-autumnal, at any or all times. The large macrophages, some of which are necrotic, are seen only in severe cases. These may contain organisms which are still within red cells.

The malarial pigment is now considered to be hematin. Formerly it was supposed to be iron-free melanin.

The Cycle within the Mosquito.—The cycle within the mosquito has been followed by several observers in the case of Plasmodium precox.

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[157] See Johns Hopkins Hosp. Bull., Nov., 1897, and Oct., 1902.

The crescents in the blood which the mosquito has ingested become circular forms. The male circulars flagellate probably in response to the same stimulus as under the microscope, *i. e.*, the lowered temperature, and fertilize the female circulars. This occurs in from 1 to 1.5 hours after the mosquito has bitten. During the flagellation of the microgametocyte the macrogamete ripens by casting off karyosomes, polar bodies consisting of chromatin, and projects a slight mound through which the microgamete has been seen to enter. The nuclear material of the macrogamete and the microgamete then unite, the new fertilized cell assumes a motile spindle form called the "vermiculus" which varies in size from 20μ up, and is found in from 40 to 48 hours after the blood has been ingested. This vermiculus actively bores its way through the epithelium of the intestine and becomes encysted between that and the elastic layer, (Fig. 135) the "tunica elastico-muscularis," which forms the membrane of the oöcyst. Its nucleus now divides rapidly (Fig. 136). This oöcyst increases in size, bulging away from the intestinal wall until it forms a pendulous tumor in the body cavity (see Fig. 135) which varies in diameter from 4.5 to 30μ or even 90μ in diameter. This stage is called the "medium zygote," or the "medium sporoblast," and is conspicuous because of the amount of pigment it contains. There may be 200 such tumors attached to the intestine of the mosquito. The protoplasm now gathers itself around the divided nuclei (Fig. 136), a process analagous to the sporoblast formation of the coccydia except that here the separation is less perfect. It is now known as a "large zygote," or a "large sporoblast." The nucleus of each of these divisions now divides into great numbers (*b*, *c*) of daughter nuclei which remain on the surface of the various daughter cysts. The protoplasm collects around each, first forming spherical cells, and these then elongate into threads lying parallel in masses over the residue of the sporoblasts. These threads are called "sporozoits." Their nuclei also become elongated. The final length of these sporozoits is about 14μ and their width about 1μ. Their protoplasm is thick, homogeneous and very refractive. All of the sporozoits of one oöcyst ripen simultaneously. Some oöcysts contain even 10,000 sporozoits while others contain but a few hundred. When ripe, the oöcyst bursts into the body cavity and the free sporozoits, moving with a bending, gliding movement as if directed by some positive chemotactic influence, finally collect in the salivary glands. Inoculated by the mosquito's bite into the blood-vessel of men, they attach themselves to, and finally penetrate into, the red blood-cells, a process actually observed by Schaudinn. They are said to stay for some time on the surface of the cell before penetrating it, and it is said that if quinine is now given they will drop off from the corpuscle. As a rule the first chill comes on about the eighth or twelfth day after the mosquito bite, yet this will depend on the number and the virulence of the parasites introduced into the circulation. Since some mosquitoes contain fully 200 of these oöcysts (of course not

PLATE V

The Blood in Tertian Malaria.

1. A hyaline form.
2. A young tertian, perhaps twelve hours old, with beginning granulation.

3, 4, 5, 6. Half grown, and slightly older, forms. Three is a large cell containing two parasites.
7. A form almost full grown.

8, 9. Full grown parasites showing division of the chromatin preceding that of the cell.
10. A small tertian parasite in a red cell showing "stippling" (Plehn's granules).

11, 12, 13, 14. Gamete (sexual) forms. 13, macrogamete in a cell with Plehn's granules.

The Blood in Quartan Malaria.

15. A very young quartan parasite.
16. A full grown form with the chromatin still in one clump.
17. A full grown form with the chromatin scattered.
18. A segmenting parasite.

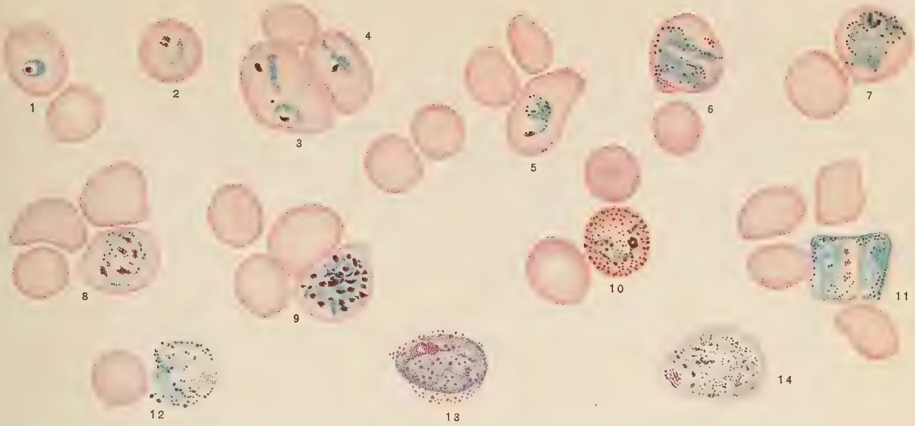
The Blood in Æstivo-Autumnal Malaria.

19. One field exactly reproduced from the blood of a case of pernicious malaria.
20. Æstivo-Autumnal hyalines showing the projection of the chromatin masses from the cells.
21. Blood platelets.
22. Æstivo-Autumnal hyalines.

23, 24. Red cells containing more than one hyaline.
25. A full grown æstivo-autumnal parasite.
26. Hyalines free in the plasma.
27. A blood platelet lying on a red corpuscle.

28, 29. Crescents.

PLATE V


1
2
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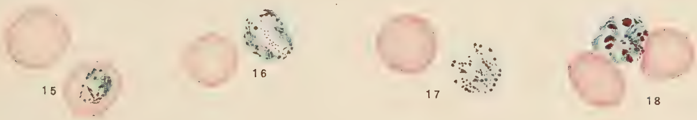
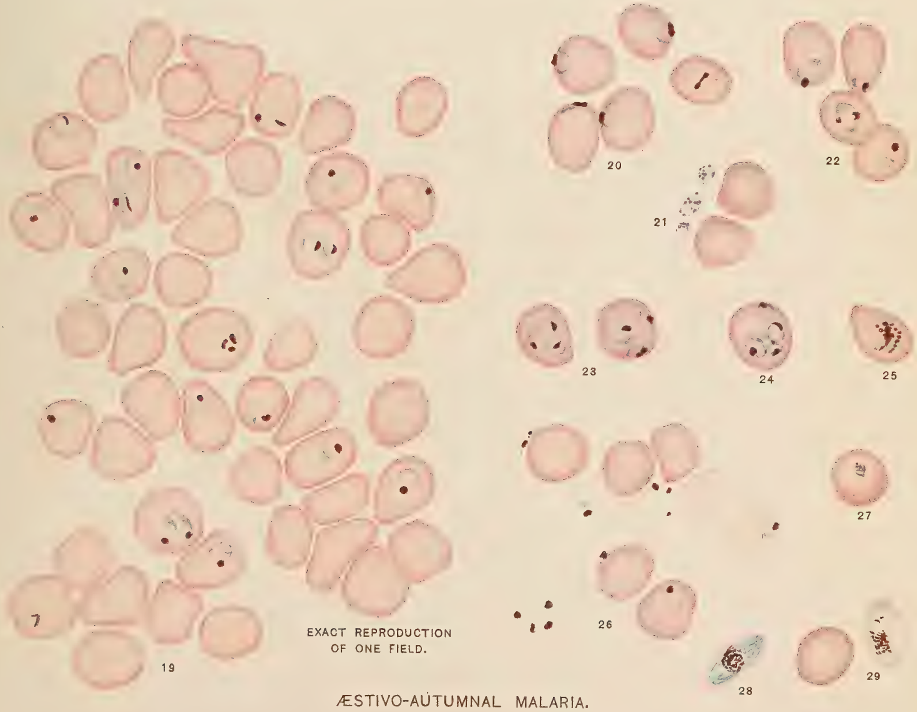
TERTIAN MALARIA.



QUARTAN MALARIA.


19
EXACT REPRODUCTION
OF ONE FIELD.
20
21
22
23
24
25
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27
28
29

ÆSTIVO-AUTUMNAL MALARIA.

STAINED WITH HASTING'S MODIFICATION OF
ROMANOWSKI'S STAIN. ALL DRAWN TO SAME SCALE.

F. S. Lockwood.

necessarily all of the same age) and since some of these contain 10,000 or more sporozoits the number of the hyalines which may be injected by one bite may be considerable.

For the tertian the optimum temperature for this cycle is 28° to 30°C., and the time 8 days; below 17° to 20° C. there is no development. The quartan form can develop at a slightly lower temperature.

The Anopheles group of mosquitoes is the only one as yet proven to be the host of the malarial organism. For a full description of these insects the reader is referred to Stevens and Christophers, and[158] Nuttall and Shipley.[159]

The Anopheles genus may be easily recognized by its "awl-shaped" attitude on a wall, since (see Fig. 137) its body forms a straight line with thorax, head and proboscis, which line forms an angle with the wall, while Culex (*b*) sits "hunch-backed," its body parallel to the wall and its proboscis at an angle of 45 degrees with its body. The genera are separated by the relative length of their probosces and palpi (see Fig. 138). Those of the Anopheles female are of equal length and scaled, while of the Culex, Stegomyia and Temiorhyncus females the palpi are short and insignificant. It is only the female Anopheles which bites. Her wings are spotted as a rule and she holds her hind pair of legs stretched out and oscillating in the air.

The Anopheles egg and larva are characteristic: the former, from its boat-like shape and lateral air-cell floats; the latter, from its attitude in the water, lying parallel with and just below the surface.

Stained Specimens of Malarial Blood.—Very thin smears of blood may be stained (see page 462) by any one of the various polychrome methylene-blue-eosin mixtures (see page 467). The fresher the smear when stained the better the preparation.

Ross has described a method which is of great value when but few parasites are present. A large drop of blood is spread on the slide over an area equal in size to a 10-cent piece. It is then dried thoroughly in the air. The slide is then covered with water and the hemoglobin laked off. Care should be taken not to wash too vigorously else all the fresh blood will be washed off. The specimen is then stained in the usual manner. Mayne [160] has modified this method as follows:

The thick smear is made by "dragging" the drops of blood sharply on the slide. Such a smear is more uniform, dries more quickly and stains more uniformly. The smear is dried in the air, then dehemoglobinized by covering it with 2 to 3% hydrochloric acid in ordinary grain alcohol until all trace of red is removed and the smear is of a clear white color. The acid alcohol is then washed out in several changes of water or in a gentle stream of running water and the specimen stained immediately.

TERTIAN (Plate V, 1-14).—The youngest hyalines consist of a mass of blue protoplasm and a clump of carmine-violet stained chromatin. Soon the "achromatic zone" appears which is the "vesicular part" of the nucleus and this may make up the larger part of the parasite. The blue protoplasm now often forms a wide crescentric ring surrounding this, with the chromatin mass between the tips of its horns but often not quite touched by them. A "milk-white" zone of Gautier often surrounds the chromatin mass but is not present at all ages nor in all of the same age. To just what part of this structure the term "nucleus" shall be applied is disputed. Stevens and Christophers, as well as many previous observers, use the term for the chromatin mass alone, while others include the chromatin mass and milk-white zone and others include also the much larger achromatic zone. The nucleus of this parasite is not specialized but rudimentary, the nuclear material being scattered in the cell or collected in 1 or more masses.

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[158] "Malaria of the Tropics," 1905.

[159] Jour. of Hygiene, vol. i, Nos. 1 and 4; vol. iii, No. 2.

[160] Public Health Reports, Apr. 29, 1919, vol. 34, p. 837.

At this point should be emphasized the necessity of studying only those specimens so well stained that the blue protoplasm and the red chromatin can be clearly seen since so many structures can resemble a hyaline; as for instance, certain degenerations of the red blood-cells and, particularly, platelets resting on the cells (Plate V, 21, 27). The platelets may have granules resembling chromatin. A platelet on a cell is always surrounded by a colorless zone, while the hemoglobin comes in direct contact with the protoplasm of a parasite; good evidence, says Ross, that the hyaline is intracellular and not adherent to the surface of the cell. (Argutinsky, Stevens and Christophers and others).

At the end of 24 hours the achromatic zone is a little larger, but the chromatin is the same in amount although now it is in a more irregular nodular mass. The milk-white zone is sometimes seen. Some forms apparently have two or more nuclei.[161] In the full-grown parasite the chromatin is broken up into clusters of fine granules occupying a large achromatic zone. Just before segmentation both achromatic zone and chromatin seem entirely to disappear, the latter later to reappear in fine granules arranged in strands and masses throughout the protoplasm, which granules congregate into 4 or 5 clusters and then separate into from 15 to 20 dense round masses. Achromatic zones now appear around each of these masses. The protoplasm collects around them as a center and the segments separate.[162] The malarial pigment at the beginning of segmentation is pushed to the periphery and, after segmentation is complete, collects in 1 or 2 masses near the center. From this it is evident that segmentation is really complete before there is any sign of it in the fresh specimen (see page 661). It is claimed by many that in the stained specimens the gamete generation can be followed from the hyaline form onward. According to Stevens and Christophers the young gamete is characterized by the position of the chromatin which lies in the center of the vacuole instead of at its edge as is the rule in the asexual forms. Some of the infected cells are filled with basophile granules which in fresh specimens appear as masses of hemoglobin suspended in a pale or even colorless stroma. "Plehn's karyochromatophilic granules," "Schügner's granules" (Plate V, 10, 13).

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[161] For evidence of conjugation, see Ewing, Johns Hopkins Hosp. Bull., 1900.

[162] For much more complex details, see Argutinsky, Arch. f. mikr. Anat. und Entwicklungsges., 1901, Bd. 59, p. 315.

Bignami and Bastianelli claim that the division of chromatin into fine granules marks the gamete even in the hyaline stage, but Lazear showed that this happens always just before segmentation.

The full-grown macrogamete (Plate V, 14) consists of an abundance of deep blue protoplasm and a small compact mass of chromatin peripherally placed and surrounded by a thin vacuole-like area. This nucleus occupies about 1/10 of the cell. The pigment is uniformly distributed. The remains of the red corpuscle often cannot be seen. In the microgametocytes (Plate V, 11) the chromatin, which is thread-like in nature and in a band arranged in a knot or skein, is centrally placed in a large achromatic zone. This parasite fills about 2/3 of the red cells. Its protoplasm, which is arranged in a ring around the nucleus, stains a grayish-green or grayish-red color and not the blue of the female form, hence the pigment is more easily seen.

QUARTAN (Plate V, 15-18).—The structure of the quartan resembles that of the tertian with the exception that the chromatin mass of the hyaline is less dense, is in fact an irregular clump of granules, and that in older stages it forms a cluster of fine granules without a distinct achromatic zone, hence is often difficult to see. This parasite forms in stained specimens a band across the cells.

ÆSTIVO-AUTUMNAL (Plate V, 19-29).—The chromatin of the æstivo-autumnal hyalines is arranged in from 1 to 3 masses or filaments. The protoplasm is more scanty than that of the other forms and remains so throughout the cycle. Characteristic of this form at a later stage is the large oval ring of protoplasm with the thicker layer opposite the chromatin mass. The young gamete forms are said to be characteristic (Maurer). They are accurately spherical and appear as rings of uniform thickness. The nucleus does not project as in the schizonts and the red blood-cell usually presents no coarse stippling (Maurer). The chromatin of the male is in a loose network which occupies the most of the cell; there is comparatively little blue staining protoplasm and the pigment is scattered throughout its body. The male crescent is somewhat kidney-shaped and is shorter and broader than is the female form. The female crescent is longer and narrower, its chromatin more compact and more or less centrally placed. It has much more blue staining protoplasm and the pigment is in a ring around the nucleus or in a clump near the center. The male circular bodies are smaller than the red cells, are perfectly spherical, their chromatin is at first in the center in a large irregular mass like a tangled thread and later is in 4 or 5 dense masses near the periphery. These are extruded as threads, the "flagella" (or microgametes). Sometimes a thin bluish envelop of protoplasm can be stained enveloping this chromatin thread. The macrogamete is 2 or 3 times as large as the male form, is often triangular in shape and has abundant blue protoplasm. Its chromatin is in a single mass at the periphery and is surrounded by a circle of pigment.

In the stained specimens (especially in sections cut in paraffin) the chromatin mass and part of the protoplasm can be seen to project from the surface of the cell, hence the belief (Argutinsky, Stevens and Christophers) that the parasite at all stages rests on the cell, not in it (Plate V, 20). Study of the fresh blood, their ameboid movements, the way they pour through a fine opening when they become extracellular, show, we think, that they are intracellular.

The following points may be emphasized: In the case of tertian and quartan malaria the infection must reach a certain intensity (250,000,000 organisms, Ross) before chills begin. Because no parasites are found does not rule out malaria, especially if the patient has been taking quinine. Fevers with long intervals are explained by the great destruction of parasites during each chill, hence several cycles must pass before enough can reaccumulate to cause a second chill.

In a case of fever the discovery of a few crescents in the blood does not mean necessarily that that particular fever is malaria, since these gamete forms may persist for months after the asexual cycle has disappeared. Such patients are malaria carriers. But in case hyalines also are found the diagnosis of malaria is justifiable, especially if the fever yields promptly to quinine. The asexual cycle is the "febrile cycle." The sexual cycle has no direct influence over the host, except that it may again start up the asexual. This explains the relapses of malaria in early Spring and especially those which follow an accident or a surgical operation even 2 years after any chance of infection has passed. Indeed Bass[163] concluded as a result of his survey of the Mississippi Delta that between 27.74% and 37.74% of all persons who have attacks of malaria during a given year will relapse during the following year; and that in the case of from 50.77% to 68.86% of all persons who have attacks of malaria during a given year it is a relapse and not a new infection.

Not all the members of the same tertian or quartan group are of exactly the same size or age and segmentation continues through at least 12 or 14 hours. This is fortunate since did they segment more in unison hemoglobinuria would probably be more common (as analogue, see Texas fever of cattle). The segmenters vary so in size that it is supposed that when the majority begin to segment all the others, including those not yet quite mature, are drawn into a "precocious segmentation" (Plate III, 16, 17). This keeps the groups at an almost equal age, for otherwise these younger forms would disturb the grouping to the degree which occurs in æstivo-autumnal malaria. This also may explain the sudden appearance of a second group in a previously single infection. That is, a few of the forms may be so young that they cannot be drawn into segmentation and so segment the following day.

The distribution of parasites in the body is variable. The æstivo-autumnal lives for the most part in the spleen, liver and bone-marrow; the same, to a less degree, is true of the tertian and still less of the quartan. It is their accumulation in various organs which gives rise to symptoms; if in the brain and medulla they may cause thrombi, hence paralyses, transient aphasias, mental symptoms and even sudden death. If in the mucosa of the gastro-intestinal tract they cause even necrosis and sloughing, hence severe vomiting and diarrhea.

Whether the severity of an infection depends solely on the number of malarial parasites in the body or whether their toxins differ in virulence is hard to answer. It certainly does not depend on the number in the peripheral blood, although in pernicious cases many organisms always are visible. That there is a soluble toxin is proven by the degeneration and even wholesale destruction of non-infected cells. This is especially true of æstivo-autumnal malaria but also of severe cases of the tertian form.

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[163] Contributions to Medical and Biological Research, Dedicated to Sir William Osler, in Honor of his Seventieth Birthday, July 12, 1910, by his Pupils and Co-workers.

TRYPANOSOMIASIS

Trypanosomiasis, or "sleeping sickness," is due to an actively motile, fish-shaped flagellate, Trypanosoma gambiense (Plate II, 21) which can be seen in the blood-plasma, moving with a screw-like motion among the red blood-cells which it scarcely disturbs. It is from 2 to 3 times as long as a red blood-corpuscle (18 to 25μ long, 2 to 2.5μ wide) with 1 flagellum anteriorly and an undulating membrane which extends its entire length.

The parasite should be searched for with a lens of medium power in fresh blood specimens, but can more surely be found by aspirating a cervical lymph gland or any edematous area. Inoculation experiments may be necessary. Sometimes many are present in the blood, but generally few. They often disappear for long periods, even a month or more, and then reappear in force, even 70 to a cover-slip specimen. It may be necessary to centrifugalize the blood to find them, in which case the speed of the centrifuge should be slow and the time short, otherwise these protozoa will be crushed in the sediment by the red cells. The symptoms of this infection seem to bear no relation to the number of parasites in the peripheral blood.

Stained with a polychrome-methylene-blue-eosin mixture the protoplasm of the body takes a blue stain, the rather large red nucleus is at about their middle, a centrosome staining intensely and in a vacuole-like area is very near the blunt posterior end and a red line of chromatin runs down the edge of the undulating membrane and terminates in the red flagellum. Various involution forms will, of course, soon be seen in a fresh specimen. The parasite contains no pigment and hence must live on the plasma. It multiplies by longitudinal fission.

For a long time it was known that similar organisms were a common and harmless parasite in the blood of fish, amphibians, birds, rats, and were an important cause of disease among horses, cattle and other domesticated animals in India, Africa, especially, and South America. The disease bore several names. The "tsetse fly disease" of South Africa caused by Trypanosoma brucei, which is carried mechanically by a fly, Glossina morsitans, which seems to play no part in its life history, is fatal to almost all domestic animals, especially the horse, mule, dog, less so for cattle, still less for the ass and least for sheep and goats. Man was, however, supposed to be immune.

The "surra" of India, a disease which attacks horses and camels especially, is caused by a parasite discovered in 1881 by Evans, which differs in no way from Trypanosoma brucei. The same may perhaps be true of the parasite of "mal de Caderas" of Central and South America which attacks especially horses.

A similar parasite was discovered in 1902 in the blood of man by Dutton and in the cerebrospinal fluid of a case of sleeping sickness by Castellani, but it was Bruce who first recognized its pathogenic importance in man. It was found in cases of sleeping sickness, but also in the blood of some apparently healthy persons. Of eighty persons in Uganda, all in apparently good health, Bruce found it in the blood of 23, but many of these died later. The present opinion is that while the human host may be apparently normal for some time, the disease will sooner or later invade the cerebrospinal fluid and cause death.

This infection can run a rather acute course, but more often it is exceedingly chronic, causing for years an irregular fever with frequent intermissions, multiple erythema, moderate anemia, marked emaciation, loss of strength, localized edema of the face, trunk and legs, enlarged spleen and swelling of the lymph glands, especially those of the posterior cervical region. Many of the cases resemble malaria. The so-called "sleeping sickness" begins when the parasite invades the central nervous system.

To demonstrate this organism in the spinal fluid this should be centrifugalized gently for fully 5 minutes, and possibly 2 or even 3 times since none may be found in the first sediment. The sediment should be examined under a well vaselined cover glass.

The parasite of man, *Trypanosoma gambiense*, can in no way be distinguished either morphologically or pathogenically from that of tsetse or surra.

There are 2 common trypanosomes which are easily distinguished from that of man: Trypanosoma theileri, which is pathogenic for cattle alone (a parasite from 2 to 3 times as long as the human form), and the trypanosome of rats, which is morphologically characteristic since its posterior end is long-drawn-out and pointed, and the centrosome is not near the end but at the juncture of the posterior and the middle thirds. This can easily be distinguished from the other forms even though they coexist in the blood. It occurs in about 10 to 30% of rats investigated in some regions, in others in even 90%.

For a good discussion of this whole subject the reader is referred to the report by Musgrave and Clegg. [164]

PIROPLASMOSIS

Leishman-Donovan Disease; Leishmaniasis.—There are 3 varieties of Leishmania: Leishmania donovani, or kala-azar; Leishmania infantum and Leishmania furunculosa sive tropica, or oriental sore.

The Leishman-Donovan Bodies (see Fig. 139), which should be studied with the highest magnification possible, are small, oval, round, or cat-shaped bodies, from 2.5 to 3.5μ in diameter, with a definite cell outline and 2 chromatin masses, a larger one, the "nucleus," almost round or oval, which stains faintly, and a smaller, bacillus-shaped "centrosome," which stains deeply and which is directed at right angles, or nearly so, to the axis of the nucleus. These 2 bodies are both in the long axis of the cell, the larger at the periphery. Many are vacuolated. The outline of the cell cannot always be seen, but these 2 masses thus arranged are distinctive. They are easily stained by the various polychrome methylene blue-eosin mixtures.

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[164] Biological Lab. Department of the Interior, Bureau of Government Laboratories 1903.

They have been found in the circulating blood only of fatal cases, but are easily demonstrated in blood obtained by splenic puncture and also in the granulation tissue snipped off with scissors from the ulcers of the disease and crushed thin in the slide. At autopsy many are found in the mesenteric lymph-glands, bone-marrow and liver.

Grown in proper media (*e. g.*, McNeal-Novy-Nicolle agar) these develop into club-shaped flagellates which have no undulating membrane.

Some lie free, but some seem to be intracellular; one or two in a leucocyte (?), from 1 to 12 in endothelial or splenic cells and even hundreds in macrophages (?). These last masses are variously interpreted. If cells, they are badly degenerated. Ross considers them to be a "matrix" in which the organisms lie and thinks that none are intracellular. Manson regards such masses as zooglia.

Their division begins in the larger chromatin mass and ends in the smaller which may begin to divide after the fragments of the larger are widely separated.

This parasite is supposed to be the cause of some cases of chronic "malarial" cachexia of the Tropics, of dum-dum fever, kala-azar, tropical splenomegaly of the tropical ulcer, Delhi boil, Aleppo button, Scinde sore, oriental sore, etc. It is a filth disease. In the Tropics it promises to prove even more important than the malarial organism.

Donovan[165] reports 72 cases with a mortality of 30.55%. Clinically one finds great enlargement of the spleen, emaciation, irregular fever, various abdominal symptoms, cutaneous hemorrhages and ulcerations.

The blood features are a moderate anemia, from 2,000,000 to 4,000,000 red blood-cells, and a leucopenia with a relative and absolute increase of the large monconuclears. The average leucocyte count is about 2,000. In a case of Neave, an 8-year-old boy with a total count of 3000, the l.m. were 67%, pmn.n. 20%, s.m. 11%, eos. 1% and myelocytes, 1%. In most cases, however, the formula is more nearly that of normal blood.

FILARIASIS

Of the various forms of filaria, the embryos of which are found in human blood, the most common is **Filaria bancrofti** (**F. nocturna**). The embryos of this filaria are from 270 to 340μ long, and from 7 to 11μ broad (see Figs. 140 and 141). They are enclosed in a sheath which is considerably longer than the parasite. The anterior end of the worm is abruptly rounded, with a six-tipped prepuce and a sharp fang; the posterior tapers off for ⅖ of its length. It has a granular median axis.

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[165] Lancet, September 10, 1904.

Their movement at first is progressive, but their anterior end seems soon to become attached to the glass and there they remain for days lashing the surrounding corpuscles. These embryos appear in the circulation towards evening. Their number gradually rises to a maximum at about midnight then diminishes towards dawn. During the day they are in the internal organs, especially the lungs. An estimated number of 40 or 50 millions of microfilaria circulating in the blood may give rise to no symptoms since their size permits them to traverse the capillaries unobstructed. These embryos apparently have no pathogenic properties whatever, but the parent worm and the immature products of conception are dangerous.

Lothrop and Pratt[166] made an hourly chart of the number of embryos present. In that case the maximum was at midnight when there were 2100 per c.cm. of blood.

The adult worms lie in the thoracic duct or smaller lymphatic trunks obstructing the lymph flow and causing lymphedema of the undrained area, therefore lymph-scrotum, varicose groin glands and various other lymph tumors. If a lymphatic varix develops near the kidney, bladder, testicle or peritoneum and ruptures, chyluria, chylocele or chylous ascites may result. This obstruction may in part be due also to the eggs, which measure from 28 to 30μ long and 15μ wide, therefore are too large to pass through lymph capillaries. They explain most cases of hematochyluria and also of elephantiasis, in which condition one seldom finds microfilariæ in the blood. In these cases it is supposed that the female parent worm located in a lymphatic vessel or lymph gland of the affected part has aborted her immature ova which, since they have a larger diameter than the healthy fully formed microfilariæ, cause embolism of the lymphatic glands resulting in great enlargement or elephantiasis of the leg, arm, breast, scrotum or vulva. The female is from 85 to 150 mm. long with a distinct neck, a head with simple minute terminal mouth, a plain cylindrical body which tapers toward the neck, and tail which is covered by a striated cuticle. The tail ends bluntly and has a small depression surrounded by 2 lips. The anus is a ventral opening on the summit of a trilobed papilla. The females are generally viviparous, each giving birth to about 1000 embryos which live probably for years in the blood. The embryos reach the general circulation through the thoracic duct.

The male is 80 mm. long, has no neck but has a tendril-like tail rolled up in 1 or 2 spirals. The esophagus is thick walled. The cloaca is ventral, with 4 pre-anal and 4 post-anal papillæ and 2 spicules.

The intermediate hosts of filaria are some varieties of mosquitoes. Some of the known efficient hosts of Filaria bancrofti are Culex fatigans, Stegomyia pseudoscuttelaris and Anopheles maculipennis. About an hour after the bite the embryos in the mosquito's stomach cast their sheaths. Some die, but others bore their way actively through the intestinal wall to the muscles, where they rest. During the next 2 or 3 days the embryo becomes larger and its alimentary tract develops. There is no increase in their number in the mosquito. On the seventh day the worm is 1.5 mm. long and perfectly developed. It now actively travels to the head and takes its position in the labium (Fig. 142), whence it enters its new host during the biting process by piercing the delicate membrane of the end of the proboscis, dropping on the surface of the skin and boring its own way through to a subcutaneous lymph channel. It requires an infection by even hundreds of these adult forms to cause a very severe case and it may be years before any symptom begins. At least 1 male and female must locate in the same lymph gland and develop simultaneously to produce embryos.

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[166] Am. Jour. Med. Sci., November, 1900.

The clinical symptoms, in addition to the various lymph tumors, are anemia, enlarged spleen and fever. In any case of lymph tumor, elephantiasis, hematochyluria, the blood should be examined. These cases are usually admitted to the surgical side, and some have been operated for inguinal hernia, the lymph-scrotum being thus interpreted. One of the chief foci in this country is Charleston, S. C. Probably there are a good many cases in this country, judging from the number found in quite widely distant cities.

Francis[167] illustrated the difficulty with which this disease is spread as follows: Nine cases of filariasis found in cities outside of Charleston showed an estimated average of only 124 microfilariæ per cubic centimeter of blood. Supposing that an infected individual harbors 124 microfilariæ per cubic centimeter of his blood and that a mosquito draws 1.15 mgms. of blood at a bite, a mosquito would then have to bite such an infected individual 7 times in order to get 1 microfilaria; or, if 7 mosquitoes bit him, only 1 of the 7 would imbibe a microfilaria. If this 1 microfilaria encountered no obstacles whatever in its cycle, either in the mosquito's stomach, in the thoracic muscles of the mosquito or in its proboscis, or on the human skin or in the lymphatics of the man, it would successfully reach a lymph-gland. Here its activities might, however, come to an end by reason of the absence of 1 of the opposite sex in that same gland.

The second set of 7 mosquito bites, if successful, might result in the deposit of a second filaria in some lymph-gland of the body but quite distant from the first one. It would not be difficult to distribute 10 infective mosquito bites over the body surface in such locations that no 2 of the 10 implanted filariæ would meet in the same gland.

If a male and female did lodge in the same gland and the female gave birth to the average 1000 embryos, then since there would be no further supply of embryos, 5217 mosquitoes would have to fill themselves of this patient's blood to get 1 single embryo. That is, for the spread of this disease, mass blood infection and mass mosquito biting both are necessary.

Filariasis occurs endemic in the Tropics where it is called "craw-craw," or the "sleeping disease." In the Fiji Islands as many as 25% and in the Friendly Islands even 32% of the inhabitants are said to carry this infection.

The blood should be examined late at night. A very thick fresh specimen is made and examined with the low power. These worms cannot be overlooked. Their motion will continue for even a week in a well-sealed specimen.

Francis recommends to obtain the blood between 10.30 p.m. and 1.30 a.m. by sticking, with a Hagedorn needle, the tip of the finger. From 6 to 8 large drops of blood are squeezed out and allowed to fall on an ordinary clean glass slide. With a fresh toothpick the blood is evenly spread over the most of the surface of the slide before it has had time to clot. The thick blood films are dried in the air in a level position, protected from flies but freely exposed to the air. As soon as they are dry the slides are stood upright, back to back, in Coplin jars containing preferably distilled water, or good tap water. To get a colorless film it should be dehemoglobinized as soon as dry. An hour usually suffices for the water to entirely remove the hemoglobin, leaving the film colorless but otherwise unaltered. If desirable the slides can be transferred to fresh water to complete the laking. The slides are examined while still wet on a mechanical stage. In such preparations the glistening microfilariæ are readily seen. In routine work where a determination of the number of individuals infected is the sole object of the investigation, examination of the wet laked films is entirely satisfactory and was followed in the government surveys.

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[167] Hygienic Lab. Bull. No. 117, June, 1919.

If the specimens remain wet 48 hours while awaiting their turn for examination, bacterial growth is likely to disintegrate the film and cause its detachment from the slide. A few drops of formalin added to the water will prevent this.

To stain the microfilariæ thick smears decolorized in distilled water as described above are made. They are sufficiently fixed to the slide dried in the air. They are then stained with Delafield's hemotoxylin for 5 minutes, steaming them over a flame, as in staining tubercle bacilli, and washed thoroughly in tap water They are next decolored with acid alcohol sufficiently to differentiate with the low power the cells of the cell column of the parasite, dehydrated in absolute alcohol, cleared in oil of cloves or xylol and mounted in Canada balsam.

The polychrome stains commonly employed in blood examinations may also be used.

Hematochyluria is due to rupture of the varicosed lymph-vessels of the bladder, these forming part of the collateral circulation which compensates for an occluded thoracic duct. Attacks of this may recur for even 18 years, each being weeks or months long and separated by intervals of months or years during which the urine is clear. The attacks come on with pain and fever, spontaneously or following exertion, excitement, etc. The sequence is hematuria, hematochyluria and chyluria. The urine contains most blood and most embryos in the early morning but most chyle after a rich meal (even 3.8% fat).[168]

Filaria Diurna (the embryos) differs little from Filaria nocturna (bancrofti), except that they remain in the circulation only during the day. The granular axis is lacking The adult form is not yet known (Filaria loa of the subcutaneous areolar tissue?).

Filaria Perstans.—These embryos which remain in the circulation day and night are about 200μ long and 4 to 5μ in width, have no sheath, a body which tapers for its posterior ⅔ and a slightly bulbous tail. They make a very active, progressive, as well as a lashing motion. The adult is found in the retroperitoneal tissue.

Other forms described in man are Filaria ozzardi (embryos small, 170 to 200μ long, without sheath and with sharp tail, no periodicity and its adult in the sub-peritoneal tissue); Filaria demarquai (embryos 200μ long, sheathless and sharp-tailed, with cephalic armature, no periodicity and the adult doubtful); Filaria megalhesi, Filaria gigas and Filaria loa.

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[168] For the blood formula, see page 561.

RELAPSING FEVER

The parasite of relapsing or famine fever of Europe is **Spirochete (ro Spirillum) obermeieri,** an organism curled like a corkscrew, from 12 to 45μ long and from 0.3 to 0.5μ in breadth (Fig 143). Its curves, from 4 to 16 in number, are sharp and regular. Its ends are pointed. It is flagellated, but there is a dispute as to the number of flagella. This organism is present in the circulating blood from the onset of the fever until the crisis when it suddenly disappears. At the beginning of the attack the parasite moves with a rapid corkscrew motion among the blood corpuscles, which are not much disturbed, but later there is simply an undulatory movement of the whole spirochete and still later merely a slight swaying motion. After the crisis, it is said, the parasites collect in the spleen. Little is known of the life history of this organism outside the human body. Relapsing fever is certainly a filth disease and the bed-bug is accused of being the agent of its natural transmission, but this is not yet proved.

Three similar organisms have been discovered.[169] These are Spirillum duttoni, the cause of the African disease; Spirillum carteri, cause of the Asiatic disease; and Spirillum novyi, cause of the American disease.

Spirillum duttoni varies in length from 15 to 45μ and from 0.2 to 0.4μ in width. It has from 2 to 6 curves. Whether it has flagella and undulating membrane is disputed. Not nearly as many of these spirilla are present in the blood during these attacks as in the case of the other spirillar fevers. Transmission to man seems to be by ticks.

Spirillum novyi is an organism whose length is from 7 to 9μ or multiples of this. It is shorter and finer than the other spirilla and has 2 or 3 sharp regular curves.

Spirilla carteri is a parasite from 12 to 16μ long and from 0.3 to 0.5μ wide. This organism causes a severe infection. Lice are supposed to spread this disease.

Trichinella spiralis was found in the circulating blood first by Herrick and Janeway,[170] who used Staubli's method. The blood, from a few drops to 10 c.c. in amount, is laked with from 10 to 15 parts of 3% acetic acid. This laked blood is centrifugalized and the sediment examined.

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[169] Mackie, N. Y. Med. Jour., Aug. 2, 1908.
[170] Arch. of Int. Med., April, 1909.

CHAPTER VI

CEREBROSPINAL FLUID

The normal amount of cerebrospinal fluid in the subarachnoid space and in the ventricles is said to vary from 60 to 150 c.c., of which from 5 to 10 c.c. or more may be withdrawn by puncture without untoward symptoms. The technic of lumbar puncture cannot be described here further than to say that a monometer, if only a long glass tube with barometer bore, should be attached to the needle, and the pressure frequently noted during each puncture. This pressure normally varies, the patient recumbent, from 120 to 180 mm. of water in the adult and from 45 to 90 in the child. The amount to be removed will depend partly on the drop in pressure caused by the loss of the first 5 c.c. of fluid removed, but more on the provisional diagnosis. In any doubtful case it is a safe rule never to remove over 5 c.c. at a time, for in patients with brain tumor sudden death has sometimes followed lumbar puncture.

It is relatively most abundant in the first years of life. In cases with traumatic fistula of the spinal canal a flow of from 1½ to 2 liters in 24 hours has been observed and in cases of cerebrospinal rhinorrhea the amount which has flowed from the nose has varied from 96 to 720 c.c. per day. These figures give some idea of the rapidity with which this fluid can be secreted. It is pathologically increased in amount in certain infectious diseases, in meningitis, always in hydrocephalus and in general paralysis of the insane. Coriat was able to obtain by puncture in alcoholic cases from 10 to 100 c.c., in dementia precox even 50 c.c., and in general paralysis sometimes over 100 c.c. In senile cases one gets even 60 c.c.

Levinson recommends that the fluid be collected in several test-tubes of uniform width, the amount removed to depend on the amount of fluid present as indicated by the pressure under which it flows.

The first few drops are allowed to escape to make sure the fluid is free of blood. Two cubic centimeters are collected in the first test-tube and is used for (1) the cytologic examination, (2) a direct smear for microörganisms, (3) cultures, (4) 1 c.c. for the permanganate test. These tests should be made at once.

From 3 to 5 c.c. are collected in the second tube. In case the pressure is not increased the contents of this tube should be used for the globulin tests, the Wassermann and the Lange tests. If the pressure is increased this tube is set aside for the formation of a pellicle.

If there is enough fluid for the third tube, from 5 to 8 c.c. are collected for (1) the second of the permanganate tests, (2) the Ross-Jones, Noguchi, Nonne and the sulphosalicylic-mercuric chloride tests, (3) Lange's test, (4) the Wassermann test, (5) the sugar content, and (6) centrifugalization for the examination for organisms.

Into the fourth tube is run any fluid to be collected over that in these three tubes.

In *color* the fluid is either absolutely limpid or has a slight yellowish color due to lutein, the pigment of blood-serum. *Xanthochromia*, or a yellowish color of the spinal fluid, is present in many cases of tumor of the cord, especially those of the cauda equina and conus medullaris, often in extradural compression of the cord, in old cases of brain hemorrhage while in some cases it is due to the trauma of a vein received at a previous lumbar puncture. The cerebrospinal fluid of patients who have had a subdural hemorrhage may be red, of jaundiced patients a greenish-yellow, while the presence of pus will give it an opaque yellow color. It may also be stained by certain drugs, as, for instance, methylene blue.

No pellicle or sediment will appear in a normal fluid properly protected from contamination.

A cloudy fluid generally means pus, while fluids from cases of tuberculous meningitis are beautifully clear at first but soon show a fine spider-web clot of fibrin strands. If a clear fluid is not kept sterile it will quickly become turbid from bacteria.

In *reaction* this fluid normally is alkaline, but even in as short a time as 10 minutes after death it may rapidly become acid. Some have claimed that it may be acid during life because of the lactic acid formed by the fermentation of decomposing cells. In 1 case lactic acid (inactive) is said to to have been present after epileptiform convulsions. Its reaction depends directly upon the reaction of the brain tissue.

The *specific gravity* of the cerebrospinal fluid, Coriat states, is normally from 1.007 to 1.010 (Halliburton, 1.006 to 1.008). While this varies much in different diseases nothing specific has as yet been determined. In general paralysis 1.009 to 1.012 are common figures; in hydrocephalus 1.008 to 1.009. These last figures are within the normal limits. In a case of spinal bifida it was reported to be 1.001.

In the cerebrospinal fluid is found from 0.04 to 0.05% of a *reducing body* the nature of which was for a long time in dispute. It was reported to be galastose, pyrocatechin or a xanthin body but lately it has been identified as dextrose. It is increased in amount by repeated tappings.

Determination of Glucose in the Spinal Fluid (Lewis and Benedict's Method). One measures 2 c.c. of the cerebrospinal fluid into a 25 c.c. volumetric flask containing 5 c.c. of distilled water. To this are added 15 c.c. of saturated picric acid solution and water up to the 25 c.c. mark. This mixture is then well shaken and filtered. One measures 8 c.c. of this filtrate into each of 2 (the 1 for a control) large test tubes of Jena glass, adds to each 2 c.c. of saturated picric acid solution and 1 c.c. of 10% sodium carbonate solution and evaporates the contents over the free flame until precipitation occurs. Three cubic centimeters of water are then added and the tubes heated again to the boiling point to dissolve the precipitate. The contents of the tubes are then transferred to two 10 c.c. volumetric flasks, cooled, the flasks filled to the 10 c.c. mark with water, shaken and filtered through cotton into a colorimetric flask and the color compared in a Dubosque colorimeter with either a glucose standard made up fresh each time or with a permanent standard solution containing 0.064 mg. of picramic acid and 0.1 gm. of sodium carbonate in 1000 c.c. of water.

Mott and Halliburton found this body absent in 12 of 14 cases of general paresis. It is usually absent in tuberculosis and in epidemic cerebrospinal meningitis. Others claim that glucose is increased in hydrocephalus (Cavazzini), diabetes (Schaefer, in which case from 0.32 to 0.35% is said to have been found) and in grave pneumonia.

Normal cerebrospinal fluids have been found to have the following composition.[1]

In 1000 parts they were:

Fixed matter	10.65 –11.00	Average	10.93
Organic matter	1.75 – 2.65	"	2.13
Mineral matter	8.50 – 9.	"	8.80
Protein	0.13 – 0.30	"	0.18
Amido acids		"	0.010
Urea	0.03 – 0.1	"	0.06
Total N	0.196– 0.198	"	0.197
[Non-proteid N	0.17 – 0.26	"	0.21]
[Creatinin	0.007– 0.015	"	0.009]
Reducing bodies	0.48 – 0.58	"	0.53
[Sugar	0.7 – 1.	"	0.7]
Organic acids		"	0.30
Chlorides	7.25 – 7.40	"	7.32
Total phosphates (as P_2O_2)	0.029– 0.031	"	0.030
Inorganic phosphates (as P_2O_5)			0.012
Organic phosphates (as P_2O_5)			0.018
Total sulphur (as SO)	0.028– 0.071	"	0.056
[Inorganic sulphur (as SO)			0.010]
[Organic sulphur (as SO)			0.046]
Nitrates			0.009
Sodium as Na_2O			4.346
Potassium as K_2O			0.251
Calcium as CaO			0.095
Magnesium as MgO			0.050

The bracketed figures are from other calculations.

Levinson, who determined the H-ion concentration in over 400 normal fluids found that immediately after the fluid was withdrawn from the body Ph ranged from 7.4 to 7.6 or exactly the same as that of the fresh blood. After standing 30 minutes Ph became 7.5 to 7.6, and in 12 hours 8.1.

He found the alkali reserve (van Slyke method) in non-meningitic fluids varied between 45.7 and 63. (or about the same as that of the blood.

The *urea* may be increased in hydrocephalus; much, even to 0.45% in nephritis and considerably in arteriosclerosis.[2]

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[1] Levinson Cerebrospinal Fluid in Health and in Disease. 1919.

[2] Widal and Froin, Gaz. des Hop., No. 122, 1904.

The chief proteid found in normal fluid is globulin. Quincke estimates the total proteid as from 0.2 to 0.5, Ricker from 0.5 to 1, and Gumprecht 0.25 gm. per liter. Halliburton found globulin alone in 13 cases and in 6 cases albumose also, while in 3, 2 of which clearly were cases of meningitis, albumin was also found. No fibrinogen is ever found normally. If a little blood-serum is added to the cerebrospinal fluid its presence will be evidenced by fibrin formation. Serum albumin is said never to be present normally. In meningocele albumose and peptone have been found.

In general paresis the total solids may reach even 2.39 p.M. and the proteids are considerably increased. There is some increase in hydrocephalus, in inflammatory conditions, in cases with stasis due to brain tumor (2 to 4 p.M.), after repeated tappings, in apoplexy and in meningitis (as a rule, 2 to 3 p.M.; but if purulent 7 to 9 p.M.).

Mott and Halliburton recommend the following QUANTITATIVE METHOD FOR TOTAL PROTEIN. The fluid is made acid with acetic acid and 2 volumes of absolute alcohol are added. It is then boiled, filtered, the precipitate dried at 110° C. and weighed.

In 8 cases of general paresis the average percentage was 0.239% and in 2 of spina bifida, 0.088%. In general paresis the proteoses and peptones were absent, but globulin and a little nucleoproteid were found.

To demonstrate nucleoproteid in the spinal fluid it is necessary to use at least 1 liter of the fluid. To this an excess of alcohol is added and the precipitate digested in water. If the undissolved residue is found to contain a high percentage of phosphorus, suggesting nuclein, the residue is washed with 0.2% HCl and heated on the water-bath at 100° C. with fuming HNO_3 and a small amount of H_2SO_4 and $KClO_3$. The residue is dissolved in HNO_3 and ammonium molybdate added; a yellowish crystalline precipitate will result.

A very good idea of the amount of protein present may be gained by the heat-acetic-acid test. A normal fluid would remain clear on standing some hours. If a fluid in which the protein is increased be boiled no cloud will appear, but on adding one drop of dilute acid a very faint white opalescence develops, which will separate in fine flocculi. If some of this fresh fluid is mixed with an equal amount of saturated ammonium sulphate solution the globulin will be precipitated. This is removed by filtration, the clear filtrate is then acidified with acetic acid and boiled. The precipitate will be albumin.

Globulin Tests.—These tests are of value only if the spinal fluid is free of blood.

a. NOGUCHI'S TEST.—One measures into a small test tube with a pipette 0.2 c.c. of the cerebrospinal fluid, adds 0.5 c.c. of butyric acid solution (5 c.c. of butyric acid in 45 c.c. of physiologic salt solution), boils this mixture for a few seconds, then adds 0.1 c.c. of a 4% aqueous NaOH solution and boils again for a few seconds. A definitely flocculent precipitate, whether of fine or coarse flocculi, which appears in from 5 to 20 minutes, is proof of an increase of globulin in the fluid. A normal fluid would remain clear for at least 2 hours or become only slightly opalescent. This is the most accurate of the globulin tests.

b. The Ross-Jones Test.—One superimposes 0.3 c.c. of the spinal fluid on an equal amount of saturated ammonium sulphate solution. The appearance of an opaque ring at the point of contact of the 2 fluids indicates an increase of globulin.

c. The Nonne-Apelt Test.—One mixes equal amounts of cerebrospinal fluid and saturated ammonium sulphate solution. A white precipitate appearing in 3 minutes is positive for euglobulin. One now filters out the precipitate, adds one drop of 10% acetic acid to the filtrate and boils this fluid. A precipitate is evidence of serum albumin.

d. Kaplan's Method.—One heats to the boiling point (twice) 0.5 c.c. of the spinal fluid and then adds 3 drops of a 5% solution of butyric acid in physiological salt solution and immediately 0.5 c.c. of a supersaturated solution of ammonium sulphate. This mixture is now set aside for 20 minutes. An excess of globulin is indicated by a thick granular precipitate.

e. Pandy's Method.—One drop of cerebrospinal fluid is added to 1 c.c. of a concentrated solution of carbolic acid (1 part of phenol crystals to 15 parts of water). A bluish-white ring or cloud will indicate an excess of globulin.

One objection to this test is that it is so sensitive that it may appear positive in the case of normal fluids.

f. The Sulphosalicylic-Mercuric Chloride Method.—One measures 1 c.c. of cerebrospinal fluid into each of 2 similar small test-tubes about 0.3 cm. in diameter. To one is added 1 c.c. of 3% sulphosalicylic acid, to the other 1 c.c. of 1% mercuric chloride solution. Both tubes are then set aside for 24 hours, at the end of which time the precipitates in the 2 tubes are compared. In the case of normal fluids the sediments in both are very slight. In all cases of suppurative meningitis, however, the precipitate in the tube with sulphosalicylic acid is even 3 times as abundant as that in the other, while in tuberculous meningitis the reverse is true. This last point has been found very valuable.

The Permanganate Test.—This test, introduced by Mayerhofer, for the total organic content of cerebrospinal fluids is a modification of the standard test used for a similar purpose in the analysis of drinking waters.

One measures with an accurate pipette 1.0 c.c. of the cerebrospinal fluid into an Erlenmeyer flask and adds 50 c.c. of distilled water and 10 c.c. of dilute H_2SO_4 (1 part H_3SO_4 in 3 parts of water). This mixture is then brought to the boiling point, 10 c.c. of *0.1 N* $KMnO_4$ solution added and the boiling continued for exactly 10 minutes. Then 10 c.c. of *0.1 N* oxalic acid are added and the *0.1 N* $KMnO_4$ run in drop by drop from a burette until the red color persists throughout the body of the fluid for several minutes. In this way one determines the amount of the *0.1N* $KMnO_4$ used up in oxidizing the organic matter.

One next determines the amount of *0.1 N* $KMnO_4$ necessary to oxidize the organic matter in the reagents used by repeating the above procedure, only without adding any cerebrospinal fluid. The result is to be subtracted from the first reading. The difference is the permanganate index.

The **saline constituents** of cerebrospinal fluids resemble those of other serous fluids.

The toxicity of the fluid has been found increased in general paresis and also after epileptic seizures. Its poisonous qualities are due to cholin and other products of nerve degeneration.

CHOLIN is a decomposition product of lecithin, the chief component of the myelin sheaths. Its appearance in the spinal fluid is evidence of nerve disintegration. It is soluble in water and alcohol, insoluble in ether and is precipitated by $PtCl_6$ as polymorphous crystals, which, however, if recrystallized from warmed 15% alcohol are regular octahedra. These crystals are insoluble in alcohol and ether, but are soluble in water.

The careful technic given by Coriat for the demonstration of cholin in the spina fluid is as follows: The proteids are first precipitated by 95% alcohol in excess and the filtrate evaporated over the water-bath at 40° C. to dryness. The residue is extracted with absolute alcohol, filtered again and this evaporated to dryness. This process is repeated several times, the temperature always being kept low. All traces of proteid and potassium salts are thus removed. The final residue, after extraction with absolute alcohol, is a syrup of a light color. This is divided into 2 fractions. The first is dissolved in distilled water, and the second in 15% alcohol. The watery solution is tested for proteid by the biuret, Millon's and other proteid reactions all of which must be negative. It is tested also for cholin by the ordinary reactions for alkaloids (phosphotungstic and phosphomolybdic acids, *et. al.*) all of which must be positive. To the alcoholic solution are then added 4 drops of 4% $PtCl_6$. It is then evaporated in a watch-glass over $CaCl_2$ to obtain the crystals. The presence of cholin may be assumed if among the former tests tannic acid gave no precipitate (thus neurin is excluded) and precipitates were obtained with phosphotungstic acid (white), phosphomolybdic acid (yellow), $PtCl_6$, $AuCl_6$, and with Lugol's (brown). On evaporating the 15% alcohol solution large yellow octahedral crystals must separate which are easily soluble in water (therefore they are not neurin). Their size, solubility in water and the fact that the aqueous solution gave the alkaloid reaction, excludes potassium. These crystals, if in a sufficient amount, may be dried and the platinum in them determined, which should be 34.8%.

In the same hydrolysis of lecithin are formed glycerophosphoric acid and stearic acid. The latter unite with the glycerol radicals to form the neutral fats upon which Marchi's stain depends.

Cholin is eliminated in the cerebrospinal fluid and the blood and glycerophosphoric acid in the urine.

The presence of cholin indicates nerve disintegration. It has been found in a wide variety of nervous disturbances: in general paresis, combined sclerosis, insular sclerosis, alcoholic neuritis, beriberi, senile dementia, delirium tremens, etc., and in amounts roughly parallel to that of proteids present. Mott considers that its presence or absence can be used to differentiate the organic from the functional nervous disturbances only in case the organic disturbance was active at the time the fluid was obtained. It is most constantly present in general paresis, in which disease Coriat found it in all of 14 cases. He found, however, no relation between its amount and the anatomical finding.

We add a table of a few analyses we had formerly made, calling particular attention to the high solid content in stasis (due to brain tumor), and to the difference between the ventricular and spinal fluids in a case of hydrocephalus, a difference which we had noted in 2 previous cases.

Cerebrospinal and Ventricular Fluids

No.	Case	Amount.	Sp. gr. (grav.).	Solids, per cent.	Proteids, per cent.	Salts and Extractives soluble in H_2O; insoluble in alcohol.	Total ash, per cent.	Extractives and salts soluble in alcohol; urea, sugar, NaCl, etc.	Total ash, per cent.	Soaps, Glycerin, fat. Cholesterin, cholin, etc., per cent.
1	Normal child	13	1007.4	......	......	......	......	......	......	......
2	Normal child	22	1008.3	......	......	......	......	......	......	......
3	Hydrocephalus cord	....	1002.	......	......	......	......	......	......	......
	Brain (fluid from)	....	1006.2	0.96	0.0991	0.2703	0.507	0.2937	......	......
4	Hernia of brain (tumor)	50	......	......	0.170	0.62	0.492	0.374	0.2092	0.023
	Later	630	1006.9	2.5132	0.1112	0.5964	0.5166	......	......	0.016
	Later (Ventricular fluids)	450	1007.7	......	......	......	......	......	......	......
5	Tumor of brain (Hernia)	200	1011.6	......	......	......	......	......	......	......
6	Gunshot wound; head	25	1009.2	......	2.664	0.7839	0.68	0.1988	......	......
7	Cerebrospinal meningitis	100	1007.	......	......	......	......	......	......	......
8	Streptococcus meningitis	25	1009.2	......	0.1628	......	......	......	......	......
9	Tuberculous meningitis	....	1018.8	......	0.066	0.5629	0.4712	0.4112	0.2445	0.0185
10	Pneumonia; meningeal symptoms	10	1006.	......	......	......	......	......	......	......
11	Paresis?	30	1008.	......	0.0597	......	......	......	......	......

Cytology.—To count the cells of a spinal fluid the tube of a leucocyte pipette is filled first to the 0.5 mark with some staining fluid, *e. g.*, Unna's polychrome methylene-blue, and then to the eleven mark with the perfectly fresh spinal fluid. This is well shaken and allowed to stand a few minutes that the cells may become stained. The shaking must be repeated immediately before the count is made. While an ordinary leucocyte counting chamber may be used, more accurate results are obtained with the special Fuchs-Rosenthal chamber which is 0.2 mm. deep and the sides of the ruled square of which are 4 mm. long. The volume of fluid bounded by this square will be 3.2 c.mm. If A = the average number of cells in the squares counted, then $\frac{10a}{3A}$ = number of cells in 1 c.mm. of the diluted fluid. A correction of 5% may be made for the slight dilution. The average number of cells in normal fluids lies between 4 and 6 cells per 1 c.mm. Above 6 is suspicious, while above 10 indicates a distinctly pathological condition.

The cells in normal fluids are small mononuclear leucocytes similar to those of the blood, together with, very rarely, a few large mononuclears.

In case the fluid cannot be examined while fresh, it may later be centrifugalized for 15 minutes and the sediment obtained spread on a slide in a round smear about 7 mm. in diameter, which is then fixed and stained. In the case of normal fluid not over 4 lymphocytes will be found in each field of a 400 magnification.

The most interesting application of the cell count of spinal fluids is in the diagnosis of cerebral lues, general paresis, and tabes dorsalis, diseases with a chronic posterior meningitis and a slight, though usually definite, spinal leucocytosis. In general paresis a lymphocytosis and an increase of the protein of the spinal fluid may be the earliest symptoms. A negative find is often of more value than a positive one. In 80 cases the counts varied from 5 to 204 cells per 1 c.mm. In 9 of these the count was under 5, in 6 over 100 (Rous). In 25 cases of this disease Cornell [2] found the count to vary from 12 to 216 cells, the average being 52. Of these cells, from 45 to 97% were small lymphocytes, from 0 to 15% (average 4%,) large lymphocytes, from 1 to 56% (average 18%) polymorphonuclear neutrophiles and from 0.1 to 5% (average 1.5%) plasma cells. In the diagnosis of cerebral lues the cell counts have thus far been of little aid. A slight spinal lymphocytosis has been found present in about half the cases of locomotor ataxia studied and the same is true of some cases of multiple sclerosis. On the other hand, in a large group of mental and nervous diseases, among which are included the psychoses of arteriosclerosis, chronic alcoholism, chronic delusional states, dementia precox, epilepsy, most manacal and hypomaniacal conditions, the psychoneuroses, uremia, hydrocephalus and cerebral neoplasm, the cytology of the fluids have thus far proved negative.

Bacteriology of the Spinal Fluid.—To determine the organism present, smears of fresh fluid should be stained and cultures made, preferably on blood agar. Among the organisms most often found in cloudy fluids are: Micrococcus intracellularis meningitidis, Diplococcus pneumoniae, Bacillus influenzæ, Streptococcus pyogenes, and several other organisms (*e. g.*, Bacillus typhosus, Bacillus coli, Bacillus paratyphosus, Bacillus mallei Bacillus pestis, the Gonococcus, staphylococci, etc.). In the clear fluid of tuberculous meningitis Koch's organism may be demonstrated.

Lang's Gold Chloride Test. The Colloidal Gold Test.—The gold chloride test has proven of great value in the recognition of early general paresis and of some value in differentiating the various types of meningitis. It is the most sensitive indicator we have of pathological changes in the spinal fluid. The reagents required to make up the colloidal solution of gold are a 1% solution of gold chloride, a 2% solution of potassium carbonate, a 1% solution of oxalic acid and a 2.5% solution of formaldehyde. All water used to make up these solutions must be triply distilled. To make a liter of the solution, 10 c.c. of the 1% gold chloride solution, 7 c.c. of the 2% potassium carbonate solution, 1.75 c.c. of the 1% oxalic acid solution and 0.83 c.c. of the 2.5% formaldehyde are added to a liter of triply distilled water in a chemically clean flask. After thorough mixing the fluid is heated to from 80° to 85° C. and kept at that temperature until a series of color changes has taken place, which runs through gradations from faint blue-green to a deep ruby-red. When the solution reaches its maximum depth of color a remarkable lightening in hue occurs within the space of a few seconds, the dark ruby-red becoming converted to a lighter shade, and when this stage is reached the reaction is finished. The solution if well made has a ruby-red color, is transparent, neutral in reaction on the day on which it is used, and 5 c.c. of it will be completely precipitated in 1 hour by 1.7 c.c. of a 1% sodium chloride solution, which shows that it is not "protected" from precipitation by electrolytes or by impurities. In addition, it must give characteristic results with known normal, paretic and luetic spinal fluids.

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[2] Am. Jour. Insan., July, 1907, vol. lxiv.

It will usually keep indefinitely without spoiling, especially if protected from the sunlight. All glassware used in making and keeping this solution and in making the test must be perfectly clean, the glass itself neutral in reaction and the pipettes accurately graduated. The vessels of glassware may be effectively cleaned by scrubbing them thoroughly with soap and hot water, rinsing for several minutes in running tap water, then immersing them in, or filling them with, the following bichromate-sulphuric acid mixture for at least one-half hour.

Potassium bichromate, powdered................	200.0
Water, distilled, up to........................	1500.0
Sulphuric acid, concentrated....................	500.0

When ready to use the cleaning fluid is emptied out, the utensils are rinsed well in running water, then with distilled water, and finally they are flushed with triply-distilled water.

It is also necessary to use pure water, which can be obtained by distilling it three times. This should be used as soon as possible after the third distillation because upon long standing it becomes unfit for use in making the gold solution. There should be no rubber connections on the still.

In using the pipettes one should not blow the fluid out since even the carbon dioxide of the breath will disturb the results. This test is a practical application of the observation by Zsigmondy that proteins in general, each to a specific quantitative degree, protect the precipitation by a sodium chloride solution of a colloidal gold suspension. In the case of pathological spinal fluids certain proteins (albumins?) may be present which precipitate the suspension while others (globulins?) protect the suspension against sodium chloride and against these other proteins.

The effect of pathological spinal fluids on the suspension will depend on the character and relative proportion of the proteids and of other substances as well. What these proteins and "other bodies" are we do not know. The results are empirical and yet very valuable. In general paresis the suspension will be completely decolorized by a spinal fluid which gives none of the ordinary chemical tests for protein.

Normal cerebrospinal fluid diluted even 1:5120 will prevent a 0.4% sodium chloride solution from affecting a colloidal gold suspension. The degree of change which the sodium chloride solution produces in the collodial gold suspension is indicated by the degree of discolorization of the suspension.

The test is made as follows: A series of 11 large test tubes are arranged in a rack and numbered. Into the first tube are measured 0.2 c.c. of cerebrospinal fluid and 1.8 c.c. of a 0.4% solution of sodium chloride. Into each of the other tubes is measured 1 c.c. of the sodium chloride solution. The contents of the first tube are now thoroughly mixed and 1 c.c. measured into tube 2. The contents of tube 2 are now thoroughly mixed and 1 c.c. measured into tube 3, and so on throughout the series until we come to tube 10 and 1 c.c. of the contents of this is discarded. Each of the 10 tubes will now contain exactly 1 c.c. of a mixture in which the spinal fluid in tube 1 is diluted 1:10, tube 2 1:20, tube 3 1:40, etc., and tube 10 1:5120. The eleventh tube will contain no spinal fluid and serves as a salt-solution color-control. One now adds to the contents of each tube 5 c.c. of the colloidal gold solution. The rack of tubes is set aside and observed from time to time. The reaction may appear in an hour but the final reading should not be made until the end of 24 hours. The tubes are to be examined by direct daylight, not by artificial light.

The change in color from the original ruby-red (0) due to different degrees of change in the gold suspension are red blue (1), violet (2), blue (3), gray (4) and colorless (5).

The typical reactions are:

Normal = 00000, 00000
Luetic = 01233, 31000
Non-Luetic = 00012, 34210
Paretic = 55555, 42100

These reactions may be expressed graphically as curves as in Fig. 144, in which *A* is the curve of normal fluid, *B* the non-luetic curve, *C* the luetic and *D* the paretic curve.

That part of the chart represented by the dilutions 1:10 to 1:160 has been called the luetic zone, and that from 1:160 to 1:1280 the non-luetic or meningeal zone. The paretic curve is the most constant of all. This in incipient paresis may be positive when the spinal fluid and blood Wassermanns are negative, the cell count within normal limits and the test for globulin negative. In the diagnosis of this disease the test exhibits its greatest diagnostic value. The luetic curve is sometimes positive in cases of tertiary lues without any symptoms or other evidence of luetic involvement of the central nervous system. The maximal color change in cases of tuberculous and epidemic cerebrospinal meningitis always occurs in the higher dilutions to the right of the midline. This test is of no aid in a differential diagnosis between the various forms of non-luetic meningitis, the tuberculous and suppurative forms.

It is of great advantage that spinal fluids to be examined by this method need not be particularly fresh.

CEREBROSPINAL FLUID IN DISEASES

Uremia.—In cases of nephritis showing the syndrome called uremia, and especially those with convulsions, the cerebrospinal fluid is greatly increased in amount and therefore on puncture will spurt from the needle in a steady stream under considerable pressure. Even 40 c.c. or more may escape before the pressure reaches normal.

The cell count may or may not be increased. The chlorides are increased even to 0.85 gms. per 100 c.c. The urea is greatly increased in amount. Lactic acid has been demonstrated.

Diabetes Mellitus.—In diabetes mellitus the cerebrospinal fluid has been found normal in appearance, amount, pressure, cell count and in every particular chemically except that glucose is increased. Foster found this even 3%. Levinson's highest figure was 0.38%. In one case this latter author found it higher in this fluid than in the blood. Acetone and diacetic acid may be present.

Chorea.—In Sydenham's chorea there is sometimes hypertension and a distinct lymphocytosis of the spinal fluid, but most observers find this fluid normal.

Epilepsy.—During a convulsion of true idiopathic epilepsy the pressure of the cerebrospinal fluid is increased. Between attacks it is normal chemically and in pressure.

Mental Diseases.—In the psychoses not dependent on some demonstrable disease, as lues, the cerebrospinal fluid is normal in every particular. In alcoholic psychoses the fluid is increased in amount and pressure while the cell count is as a rule normal.

In **hydrocephalus** the fluid, while increased in amount, is practically normal in every other way.

In cases with recent **cerebral hemorrhage** the cerebrospinal fluid is red from the presence of blood and later yellow from the presence of modified hemoglobin. The cell count and proteid content will depend on the amount of blood present.

In **tumors of the brain** the fluid may spurt through the needle under high pressure. In such a case the flow should be checked at once and not over 5 c.c. in all removed, for the higher pressure in the cranial cavity when that in the spinal canal becomes lower may crowd the medulla into the foramen magnum and cause death. When the tumor is so located that it causes stasis of the flow of this fluid its protein content and cell count may be much increased.

Compression of the Cord.—In tumors of the cauda equina and conus medullaris the spinal fluid often shows a yellowish color (xanthochromia), its proteid is greatly increased and a lymphocytosis is present (Froin's syndrome).

If the tumor is at a higher level there may be no lymphocytosis but an increased amount of globulin and usually a xanthochromia.

Acute Encephalitis.—Excluding that which is a part of meningitis or poliomyelitis and that due to the acute infectious diseases, encephalitis is a condition easy to assume but difficult to define. In those cases with clinical symptoms suggesting such a condition the spinal fluid usually is normal or slightly increased in amount and the cell count perhaps a little increased, but otherwise it is normal. In some cases of epidemic encephalitis a slight increase in the cell count (from 10 to 100) and a slight increase in globulin was reported but in other cases this fluid was reported normal.

Meningism.—The term meningism is applied to that large group of cases, most of them of the acute infectious fevers in children, with many clinical symptoms of meningitis but with a spinal fluid sterile and clear, usually with normal cell count and normal globulin, but sometimes under increased pressure.

Epidemic Cerebrospinal Meningitis.—In epidemic cerebrospinal meningitis, the spotted fever and epidemic cephalalgia of older writers, the spinal fluid is increased in amount and pressure (unless the exudate has become so thick, literally buttering the cord, that none could possibly flow through a needle. It is of interest that even such an exudate, demonstrated by laminectomy, may disappear entirely, leaving the meninges quite free from any evidence of previous inflammation.) The pressure of the fluid is as a rule increased from 300 to even 800 mm. of water. Its color may vary from a slight greenish turbidity to that of cream. On standing the pus by settling forms a sediment of varying amount.

The protein content is greatly increased, even to 0.7%, and the sugar is greatly diminished. The cell count may reach several thousand per c.mm. the great majority of which (90 to 98%) are polymorphonuclear finely granular cells. In the smears may usually be found a few, minute, Gram-negative, biscuit-shaped diplococci, the most of them intracellular. This memingococcus grows on most culture media and in that way differs from other diplococci of similar morphology which also are Gram-negative.

From some cases both the meningococcus and pneumococcus may be isolated.

Micrococcus Intracellularis Meningitidis.—This organism (Fig 145) closely resembles the gonococcus in morphology. While some are extracellular, its most typical appearance is as intracellular biscuit-shaped diplococcus which varies noticeably in size. The meningococcus is Gram-negative, is stained easily with the common bacterial stains and can be grown on many of the common culture media.

Pneumococcus Meningitis.—In pneumococcus meningitis the fluid escapes under high pressure, is milky-white as a rule and has not the greenish color almost constant in cases of the epidemic form, than which also it is more purulent, is richer in fibrin and deposits a more abundant sediment. The cell count is high, the leucocytes chiefly polymorphonuclear finely granular cells and the organism (Micrococcus pneumoniæ) more numerous in the smears and easily grown in cultures (Fig. 146).

Streptococcus Meningitis.—The fluid in streptococcus meningitis resembles that due to Micrococcus pneumoniæ except that streptococci can be demonstrated in smears and in cultures. Both the hemolytic and non-hemolytic forms may be found.

Influenza Meningitis.—The fluid is a very thin pus containing Bacillus influenzæ in great numbers. (Fig. 147).

Typhoid Meningitis.—F. M. male, aged 20, No. 5146, was admitted August 2, 1917 and died August 7, 1917. On admission he was clearly typhoid in condition, evidently in the second week of this disease, with apparently a meningeal involvement. August 3rd the spinal fluid was negative in appearance and on examination except that it gave a one plus globulin test and had a cell count of 6 per c.mm. On August 4th the cell count was 15 and the culture of the fluid was positive for Bacillus typhosus. On this day also the blood Widal was positive and the leucocyte blood count 7600. August 5th Bacillus typhosus was found in both spinal fluid and blood cultures. August 7th the blood leucocyte count was 36,600 and the spinal fluid purulent. Stained smears of the spinal fluid showed it to contain many bacilli.

Tuberculous Meningitis.—In tuberculous meningitis, the acute hydrocephalus of former writers, the fluid early is under greatly increased pressure, from 300 to 700 mm. of water (from 240 to 550 mm.; average 365 mm.)[3] but this slowly decreases as coma develops. It usually is quite normal in appearance when drawn, is crystal clear even though it may contain even 500 cells per c.mm., but it develops a foam when shaken and on standing a fine web-like clot of fibrin appears which alone is almost sufficient for diagnosis. The specific gravity is as a rule about 1.006. There is a definite lymphocytosis of from 80 to 952 cells per c.mm. although early many of the cells may be polymorphonuclear finely granular leucocytes.

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[3] Rous, Am. J. Med. Sc. 1907.

It is of importance in diagnosis that in this disease the count usually is above 100 while in lues of the central nervous system it is usually below 100. The protein is greatly increased in amount, ranging between 0.1 and 0.2 %; the permanganate index is above 2; the chlorides are reduced, are usually below 0.6%; the glucose as a rule varies between 0.5 and 0.6%; and the H-ion concentration of the fresh fluid averages from 7.4 to 7.6.

The colloidal gold test shows a discoloration of tubes 5-6-7-8 and gives a curve of which 0001233210 is typical.

For a positive diagnosis one must demonstrate Bacillus tuberculosis. The fresh fluid, diluted with an equal volume of alcohol to lower its specific gravity, may be centrifugalized vigorously and smears made from this sediment. If a reticulum has formed this pellicle will contain the most of these organisms and smears made of this will often demonstrate the organism. Or, the fresh fluid (or the fluid after any clot present has been digested) may be injected into a guinea pig.

Syphilis.—In every case of syphilis, no matter how early, the spinal fluid should receive careful attention. There is indeed evidence that the so-called neurasthenia present in the secondary stage may indicate an early involvement of the nervous system. Certainly no case should be discharged from treatment as cured unless the Wassermann and cell count of the spinal fluid are negative as well as the Wassermann of the blood. Many relapses in cases clinically and seriologically cured are due to reinfection of the vascular system from the cerebrospinal system.

Cerebrospinal lues, tertiary syphilis of the central nervous system, is accompanied by an increase in the amount of spinal fluid, an increase in the protein, in the cell count, by a positive Wassermann reaction and a fairly typical colloidal gold curve.

In general paresis the examination of this fluid is most important. This will usually demonstrate an increase in amount, a lymphocytosis of moderate grade, an increase in the globulin, a typical colloidal gold curve and, in 90% of the cases, a positive Wassermann reaction.

Acute Syphilitic Meningitis.—In acute syphilitic meningitis the fluid is slightly increased in amount and so is under slightly increased pressure, is clear or slightly opalescent, the globulin tests are positive, there is a definite pleocytosis, (the counts varying from 100 to 600 per c.mm., 60 to 80% of which are lymphocytes), the Wassermann test is positive and the colloidal gold test gives a curve which is luetic in character.

The fluid will be sterile on culture for bacteria but Spirochete pallida has been demonstrated in the sediment.

This condition should be excluded in all cases with clinical symptoms suggesting menginitis and with fluid sterile on culture.

Acute Anterior Poliomyelitis.—In poliomyelitis the spinal fluid is increased in pressure (from 300 to 700 mm. of water) and in amount, since from 20 to 50 c.c. are easily obtained on puncture. Usually it is colorless, but sometimes slightly opalescent, and foams considerably on shaking. The globulin is slightly increased as a rule but not always.

The cell count is increased, the pleonucleosis manifesting itself in the pre-paralytic stage and lasting from 14 to 16 days after the appearance of the paralysis. The count is highest during the first week of the paralysis. Very early the polynuclear cells may predominate, but later the percentage of small mononuclears will vary from 60 to 90%. The spinal fluid is sterile by ordinary cultural methods, while some authors have found a coccus which grows best under aerobic conditions on a 1% glucose broth medium. (Nuzum, Rosenow). Flexner and Lewis found a filterable virus.

CHAPTER VII

EXAMINATION OF VARIOUS FLUIDS

Among the various fluids which may deserve the attention of the diagnostician are the plasma, serum, lymph, the various transudates and exudates, the cystic fluids, the synovial and the amniotic fluids.

Specific Gravity.—The specific gravity of a fluid may be determined with an accurate aerometer (see page 429) if there is sufficient quantity at hand but more accurately gravimetrically. The figures given in the following pages were determined by this latter method.

Dried Constituents.—The dried constituents of a fluid are determined by measuring or weighing into a weighed glass dish with a ground glass stopper from 10 to 30 c.c. of the fluid in question. This is evaporated over a water-bath and then *in vacuo* over sulphuric acid. It is then dried to constant weight at temperature not over 110° since urea or other fragile bodies usually are present and would be broken down by the heat.

Proteids.—Among the proteids which may be met with in body fluids are serumalbumin, serumglobulin and, in some, fibrinogen. The albumoses are rare. True peptone is said never to occur, while the glyco-proteids and the phospho-proteids may be met with, *e. g.*, in the cystic fluids. To isolate the proteids it is first necessary to remove any organized structures. This may be done by sedimentation, centrifugalization or by filtration through paper or Kieselguhr. Fibrin will be evident to the naked eye, will disappear rapidly in artificial gastric juice, and undergoes a glassy swelling on the addition of 0.1% HCl.

Albumin, Globulin, Fibrinogen.—From 20 to 50 c.c. of the fluid are mixed with an equal amount of saturated $(NH_4)_2SO_4$ and allowed to stand 1 hour. The amount chosen should not contain more than 0.2 to 0.3 gm. of proteid for each precipitate. It is then filtered through a weighed filter and the precipitate washed with half-saturated $(NH_4)_2SO_4$ until the filtrate gives no cloud with acetic acid and K_4FeCN_6.

(*a*) The filtrate is boiled, acetic acid added until it is faintly acid, then boiled again and filtered through a weighed ashless filter. The precipitate is then washed with hot water, then with alcohol, then with ether and brought to a constant weight at 120° C. It is then ashed and the weight of this subtracted.

(*b*) The precipitate on the filter paper is heated to 110°, washed with hot water, then with alcohol and ether, dried to constant weight and its ash subtracted. This will equal the weight of the serum globulin and fibrinogen.

For the determination of both together see page 698.

Glyco-Proteids and Phosphorus-Containing Proteids. *A*. Mucin.—In general, to isolate mucin 100 c.c. of fluid are diluted if necessary with water, precipitated with acetic acid, filtered and the precipitate washed with water acidulated with acetic acid. The precipitate is then dissolved in weak alkaline water and reprecipitated with acetic acid.

A part of this precipitate is boiled on the water-bath with dilute mineral acid (HCl), filtered and the filtrate tested for sugar to demonstrate the reduction of copper. Considerable boiling may be necessary and the reduced copper may be seen only after the fluid is cold and the precipitate has settled.

Mucin is a glyco-proteid of a stringy consistency and insoluble in acetic acid even in excess. Mucoid is similar in nature, but differs in some physical characteristics. A sharp line cannot be drawn (see page 219).

B. Phosphorus-containing Proteid.—A part of the precipitate is examined for organic phosphorus. It is ashed, the ash dissolved in dilute HNO_3, heated to boiling, concentrated somewhat and then ammonium molybdate added in excess. A yellow color and then a yellow precipitate which forms most readily at 40° C. is evidence of phosphoric acid. Or, the precipitate may be dissolved in HCl, made strongly alkaline with ammonia and then magnesium mixture added. The white precipitate of NH_4MgPO_4 indicates the presence of phosphoric acid.

If the reaction for phosphorus is faint the test has no meaning since all of the phosphate and lecithin cannot be washed from the mucin precipitate.

If organic phosphorus has been found, a part of the original precipitate is dissolved in NaOH, then HCl added, boiled to clear solution, supersaturated with ammonia and then precipitated with $AgNO_3$. A flocculent cloud of the silver salt of the nuclein base indicates a nucleo-proteid. If none forms, the phosphorus body is a paranucleo-proteid.

Fat, Lecithin and Cholesterin.—To from 20 to 50 c.c., weighed or measured, of the fluid to be examined for fat, lecithin and cholesterin are added from 3 to 4 volumes of absolute alcohol. The specimen is then allowed to stand until the next day during which time it is repeatedly stirred. It is then filtered and the precipitate washed with absolute alcohol and placed in the cylinder of a Soxhlet ether-extraction apparatus. The alcohol filtrate is now neutralized and evaporated at 60° C. The residue is taken up with alcohol and ether and re-evaporated. The residue is then taken up with ether and poured into the flask of this same Soxhlet apparatus. The precipitate is then extracted for hours. The ether extract is evaporated, the residue taken up in water-free ether, the filtered solution evaporated in a weighed beaker and dried to constant weight *in vacuo* over sulphuric acid. This residue is made up of fat, lecithin and cholesterin.

The residue is now dissolved in alcohol, alcoholic KOH added, warmed on the water-bath for one hour and then evaporated to dryness. The fat is now in the form of soap and glycerin. To it is now added water (not too little) and it is shaken out several times with equal volumes of ether.

This ethereal extract is distilled to small volume, then evaporated in a weighed beaker to dryness. The residue contains soap and cholesterin. The soap may be washed out with small portions of cold alcohol slightly acidulated with HCl. The cholesterin left is dried at 80° C. and weighed.

The alcohol washings with the soap are added to the water extract of the previous separation which now contains all the lecithin-phosphorus. This fluid is evaporated, the residue ashed and its phosphorus determined.

(Distearyllecithin contains 3.84% of phosphorus and dipalmityl-lecithin, 4.12%.)

Leucin and Tyrosin.—A fluid to be examined for leucin and tyrosin is worked with as fresh as possible. All albumin should be removed by heat and acetic acid, or by precipitation with from 3 to 4 volumes of alcohol, then heated on the water-bath, cooled and filtered. The alcohol is then removed by evaporation. The filtrate is precipitated with neutral, then with basic, lead acetate, avoiding carefully any excess, and filtered. The lead is removed from the filtrate with H_2S, the filtrate then evaporated and examined for crystals (see page 254).

Succinic Acid, $CH_2COOH.CH_2COOH$.—Traces of this acid are met with in many animal fluids; sometimes in the fluid of hydrocephalus and hydrocele, in larger amounts in echinococcus cysts and in wool-fat. It is formed by the bacterial decomposition of proteids and sugar. It frequently occurs in acid milk in the intestine, in putrid pus and whenever alcoholic fermentation of sugar is in progress.

Any fluid to be examined for succinic acid is freed of albumin by heat and acetic acid. (If it be urine which is tested the albumin is first removed, the urine perfectly precipitated with baryta water and the excess of this removed by H_2SO_4.) The filtrate is next evaporated to a residue, acidified with HCl and extracted repeatedly with ether. The ether is evaporated off, the residue taken up with a small amount of water and allowed to stand until crystallization. Or, the watery filtrate may be heated to boiling, nitric acid added drop by drop until it takes a slight yellow color and then evaporated. If no crystals form a portion of the residue is fused in a test-tube with ammonia and zinc dust. If a match-stick wet with strong sulphuric acid is held at the mouth of the tube the red color of pyrrol would indicate succinic acid. If this test is used, however, hemin and the indol-derivatives must be excluded. These latter will give the reaction on heating alone and hemin on heating with zinc dust alone.

Lactic Acid, $C_3H_6O_3$.—Of the 3 modifications of lactic acid the inactive, or the lactic acid of fermentation, occurs oftenest in the stomach and intestine of man. The dextro-rotatory form, or sarcolactic acid, may be demonstrated in the muscles, blood, pericardial fluid, aqueous humor and intestinal contents. It occurs also in the urine in acute yellow atrophy and phosphorus poisoning, liver cirrhosis, after respiratory distress, severe exercise and before death. It occurs in pathological transudates often in abundance, in the bones in osteomalacia and in the sweat in puerperal fever. The levo-rotatory form has never been found in the body.

The fluid to be examined for lactic acid is, if necessary, made faintly acid with dilute H_2SO_4 and is boiled and filtered to remove any albumin. Baryta water is added to the filtrate as long as a precipitate forms and the excess of the barium removed with CO_2. The filtrate is now evaporated to a thin syrup without heating it above 70°, in order to avoid the development of a brown color. About ten volumes or more of absolute alcohol are then added slowly to the syrup, this well stirred, allowed to stand for some time and the alcohol then poured off. The residue is dissolved in a little water and this procedure is repeated once more with alcohol.

The combined alcoholic solution is poured off, filtered, the alcohol distilled off, leaving a thin syrup which is digested on the water-bath at a moderate temperature to drive off the alcohol and then cooled. To the resulting thin syrup is added an equal amount of dilute phosphoric acid. It is then brought into a large flask and shaken out with a large amount of ether which will gradually take up the lactic acid. The ether must be frequently renewed. The united ether extracts are then filtered clear, the ether distilled off, the residue dissolved in water, boiled for some time with an excess of $ZnCO_3$, filtered, washed with hot water, evaporated to a small volume on the water-bath and allowed to stand until the zinc salt of lactic acid crystallizes out. Alcohol is then added to the mother liquid which is then allowed to stand longer and another mass of crystals is obtained. The zinc salt is dissolved in hot water and the zinc precipitated by H_2S. The filtrate is then evaporated to a syrup containing the lactic acid.

It is quite impossible to recognize lactic acid from its crystalline form or by Uffelmann's test alone.

Inosite $C_6H_6(OH)_6$.—Inosite is found in traces in the urine of perhaps every normal person. It certainly is present in cases with polyuria and therefore in the urine of some patients with diabetes and nephritis. It is found also in the contents of echinococcus cysts.

To demonstrate inosite the fluid is freed from albumin by heat and acetic acid, the phosphates are precipitated by baryta water, the filtrate is evaporated and the creatinin allowed to crystallize out by boiling with from one to four volumes of alcohol. If a heavy precipitate results which sticks to the glass the fluid is simply decanted, but if flocculent, it is filtered through a heated filter and then allowed to cool. The fluid then stands for 24 hours. If inosite is present crystals will form which may be filtered out and washed with cold alcohol. The alcohol precipitate mentioned above may be dissolved in boiling water, from 3 to 4 volumes of hot alcohol added and the above procedure repeated to recover the inosite therein contained. If no crystals form one adds ether, little by little, to the clear alcoholic filtrate until a slight milky cloudiness results which does not disappear. The specimen is then allowed to stand for 24 hours. All the inosite will be precipitated as mother-of-pearl plates. In case urine is the fluid examined, one first precipitates with baryta water and precipitates the filtrate, after heating, with PbAc, avoiding an excess. The specimen is then allowed to stand, is filtered, the precipitate washed, suspended in water, decomposed by H_2S and the filtrate evaporated. One then proceeds as above.

Inosite crystallizes in rhombohedral crystals which melt at 225° C., are soluble in 1:75 of water and soluble in alcohol or ether. It does not ferment, nor does it rotate the plane of polarization; it dissolves $Cu(OH)_2$ without reduction, is precipitated by PbAc and does not give crystals with phenylhydrazine.

Scherer's Test.—A small amount of the precipitate is evaporated almost to dryness with nitric acid on a platinum-foil. To the residue are added ammonia and 1 drop of $CaCl_2$ and the evaporation then continued to dryness. A beautiful rose color is the result. The crystals must be quite pure to give a positive test.

Seidel's Test.—This test is similar to the above with the exception that strontium acetate is used instead of calcium chloride and the positive result is a green color with a violet precipitate. This test is positive if 0.3 mgm. of inosite is present.

Allantoin, $C_4H_6N_4O_3$.—This body is found in the urine of the new-born child. A slight trace is said to occur in all normal urines and especially that of pregnant women. It occurs in some ascitic fluids, as in liver cirrhosis and in certain ovarian cysts.

To demonstrate allantoin the albumin of the fluid to be examined is removed by heat and acetic acid. The fluid is then precipitated with $Hg(NO_3)_2$, the precipitate washed, suspended in water, decomposed with H_2S and filtered. A little ammonia is added to the filtrate and the whole evaporated on the water-bath to a small volume. The clear fluid is then precipitated with ammoniacal $AgNO_3$. (Since the precipitate is soluble in an excess of ammonia this must be avoided.) After the specimen has stood for some time the silver salt of allantoin is collected on the filter, is washed, suspended in water, decomposed with H_2S, the filtrate evaporated and allowed to crystallize.

The Loewy method is recommended for the demonstration of allantoin in the urine. The faintly acid urine is precipitated with mercurous nitrate (which is dissolved in as little acid as possible plus some metallic mercury), is filtered and the precipitate well washed. The filtrate is then precipitated with H_2S and filtered, the filtrate warmed to drive off this gas, MgO then added and the whole precipitated with $AgNO_3$. This precipitate is filtered, washed, suspended in water and decomposed with H_2S while warm. This filtrate is evaporated to dryness, the residue extracted with hot water and when cold precipitated with $Hg(NO_3)_2$. This precipitate is well washed, decomposed with H_2S, the filtrate evaporated to a concentrated solution whereupon the allantoin will crystallize out in glistening prisms, which are odorless, tasteless, soluble in 160 parts of cold water and more in warm, and insoluble in absolute alcohol or ether. For its identification the silver salts are studied, which are obtained by precipitating a concentrated solution by ammoniacal $AgNO_3$. This precipitate is soluble in excess of ammonia. The white flocculent precipitate on standing becomes granular. If dried at 100° it gives an easy reduction of silver. The silver salt of the allantoin dried *in vacuo* gives on fusion 40.71% of silver.

Quantitative Analysis of Serous Fluids.—The method given by Thierfelder[1] is the one we have used. It is fairly satisfactory as a routine preliminary method for getting an idea of the general composition of a fluid, but this determined it is better to determine each substance or group in a special portion. We give the outline of the analysis.

To from 20 to 50 c.c. of the fluid, measured or weighed and freed from the formed elements by filtration, are added from 3 to 4 volumes of absolute alcohol. This is allowed to stand a few hours, filtered through a weighed filter and washed a little with alcohol. This will give Filtrate I. The precipitate on the filter paper is then washed with boiling alcohol, again and into the same flask with ether and then again with alcohol. This gives Filtrate II. The precipitate is now washed with boiling water thoroughly. This gives Filtrate III. The precipitate remaining on the paper will contain the proteid, a few salts, hemoglobin if any be present, but not much of the other pigments. It is now washed once more with alcohol and after the precipitate next to be mentioned has been added to it dried at 120° C. to constant weight. It is then ashed and the weight of the ash subtracted from that of the precipitate; this gives the weight of the proteids.

Filtrate I is evaporated at a temperature not above 60° C. To this residue is then added Filtrate II. The residue and fluid are well mixed and the fluid decanted through a dried and weighed paper. It is then washed repeatedly with absolute alcohol, then with ether and decanted each time, but none of the precipitate is allowed to get onto the paper (*B* 3). Onto the residue is now poured Filtrate III, the residue is well mixed and then all filtered through the same filter-paper, but into another flask from that which has received the above mentioned decanted fluids. The precipitate is now brought on the paper and washed with water. This precipitate is dried and added to the above mentioned proteid precipitate, since it contains that amount of proteid which was lost in the first precipitation. The watery extract of the above is evaporated in a small weighed porcelain dish in a water-bath, dried at 110° to 115° C. to constant weight and washed.

B 2 is burned at a moderate heat, ashed, and the weight of the ash determined.

B 3. This alcohol-ether extract is evaporated on the water-bath at a temperature not above 60° C. and dried *in vacuo* over H_2SO_4. To the residue is added ether and it is filtered into a flask through a small paper, washing repeatedly with ether. This will contain the urea, sugar, soaps, sodium chloride, fat, lecithin, cholesterin and the cholesterin ester.

B 3*a* is the residue of the above. This is washed from the beaker and paper into a small weighed porcelain dish, evaporated, dried at a temperature from 110° to 115°, fused at a moderate heat and weighed.

B 3*b*. This ethereal extract is treated as on page 557. It contains fat, cholesterol, lecithin, cholin, etc.

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[1] Hoppe Seyler, Chemische Analyse, 1903.

The Fat Percentage of Milk may be determined by mixing well in a large Babcock centrifuge tube

15.0 c.c. of well mixed milk
1.5 c.c. of amyl alcohol and
1.5 c.c. of conc. HCl.

and then added 10 c.c. of concentrated H_2SO_4. The specimen is again well mixed and the Babcock tube filled to the upper mark with 50% H_2SO_4. It is centrifuged for 3 or 4 minutes and then the fat percentage read off.

To determine the milk proteids one estimates the total nitrogen (see page 107) and multiplies by the factor 6.37.

To count the bacteria in milk one dilutes the milk 1:10 and 1:100 and plates with agar-agar using from 0.1 to 1.0 c.c. of the diluted milk. The plates are kept for 1 night in the incubator at 37° C. and the colonies counted on the following day.

TRANSUDATES AND EXUDATES

Although the pathologists believe that no sharp distinction should be attempted between exudates and transudates so far as the mechanism of their origin is concerned, yet the clinical chemist is forced to differentiate between them on the basis of their physical and chemical properties. In some measure at least a transudate is less the result of an inflammation than is an exudate. The transudates resemble lymph; they contain few formed elements and almost no fibrin. Their proteids are serum albumin, serum globulin and a little fibrinogen, yet not enough of the last to coagulate these fluids spontaneously, although they will coagulate if blood is added. The exudates are richer in formed elements, coagulate spontaneously, contain the so-called "nucleo-albumin" and a mucoid substance. Some claim that fluids whose specific gravity is under 1.018 are usually transudates; and those in which it is over 1.018, are exudates. The list of extractives which may be present in these fluids includes urea, glucose, creatinin, uric acid, lactic acid, inosite, succinic acid and allantoin. More rarely one finds leucin, tyrosin, bile acids and pigments, fat, lecithin and cholsterin.

The presence of "nucleo-albumin" or, better "euglobulin," is valuable in distinguishing these two classes of fluids. If a few drops of acetic acid are added to a clear exudate a cloud of varying depth, but usually quite dense, will form. It is rather soluble in excess of the acid. The cloud in transudates is very much lighter.

Peritoneal Fluid.—In cachexia and hydremia this fluid is slightly colored, of a milky opalescence, does not clot spontaneously, has a specific gravity of from 1.005 to 1.015 and contains almost no cells.

In chronic passive congestion the specific gravity is usually lower than 1.020. Sometimes it contains 35 gms. per liter of proteid. In cases of cancer of the peritoneum the fluid is turbid with cells, has a dirty grayish appearance, a high specific gravity and often clots spontaneously. The serous fluid present in inflammations is of a straw or lemon-yellow color, is somewhat cloudy from the formed elements, coagulates spontaneously, contains 30 gms. or more per liter of proteid and has a specific gravity of 1.030 or above. Mucoid substance is perhaps always present, which may be demonstrated by removing the albumin by heat and then precipitating the filtrate by alcohol. A precipitate forms from which one can split off a reducing body.

In the ascitic fluid may also be determined urea, uric acid, allantoin, xanthin, creatinin, cholesterin and sugar.

The ascitic fluids we have examined (none were very acute cases) had a specific gravity varying from 1.0055 to 1.0198, the solids from 1.3 to 4.5 gms. per liter and globulin 40 to 50% of the proteid.

Pleural Fluid.—Physiologically there is not enough pleural fluid present for an analysis. The pathological fluid may be of any degree of serous, sero-purulent, purulent or hemorrhagic quality. In hydrothorax the specific gravity is lower than 1.015 as a rule, the albumin from 10 to 30 gms. and the fibrinogen hardly 0.1 gms. per liter. In pleurisy the exudate has a specific gravity above 1.020 as a rule, albumin 30 to 65 gms. and the fibrinogen 1 gm. per liter.

In 9 of our cases the specific gravity of the fluids varied from 1.0122 to 1.0252 and the solids from 3.12 to 7.926%. The more acute the case the higher the figures. The amount of total proteid varied from 2.837 to 6.529%, of which from 39 to 64% was globulin. The amount of globulin depended on the acuteness of the case. The acetic acid precipitate was markedly more in the acute inflammatory cases than in the transudates. It is interesting what little difference the clotting makes. In a case of acute tuberculous pleurisy before the fluid clotted its specific gravity was 1.0221 and the globulin 2.875%; after clotting (densely) these figures were 1.0217 and 2.376% respectively. To clear these fluids with the centrifuge is better than with Kieselguhr.

In typical cases of pleurisy with effusion, and these are usually tuberculous in character, the fluid is yellow or faintly greenish-yellow in color, with a specific gravity of at least 1.018 and an albumin content of 4% or more. On the addition of acetic acid an abundant precipitate falls. The fluid contains much fibrinogen and therefore coagulates rapidly, forming sometimes small clots, or the entire mass of fluid may become a gelatinous clot (rarely must a little blood be added to aid clotting). Microscopically it contains leucocytes and a few red blood-cells. In the majority of cases the most of the leucocytes are small mononuclears.

Cytodiagnosis is the diagnosis of the etiology of an effusion based on the formula of its cell count. The fluid to be examined should be received into a sterile flask about ⅓ full of 0.85% sodium chloride solution and containing also 1% sodium citrate. This fluid is centrifugalized and the smears made from the sediment are stained in the same manner as are blood smears. One can recognize in these smears the following leucocytes (Fig. 148): small mononuclears, polymorphonuclear finely granular cells, polymorphonuclear coarsely granular cells, basophiles and endothelial cells. The latter demand special attention. Some may be the large mononuclear leucocytes of the blood (the so-called endothelial leucocytes) while others occur in sheets as though stripped directly from the pleural membrane. Some are phagocytic. All of these leucocytes are apt to have pycnotic nuclei and a vacuolated, degenerated protoplasm. Widal claims that if the majority of the cells in an exudate are finely granular the case is one of pyogenic infection; if the small mononuclears, it is one of tuberculous pleurisy, while in pleurisies of mechanical origin the endothelial leucocytes will predominate. But the formula varies not only with the etiology but also with the intensity of the infection and with the duration of the effusion. We may have a transitory leucocytosis in cases of early tuberculous pleurisy; we may also in such a case have a secondary infection of the pleura with pyogenic organisms. Many effusions which are clearly non-tuberculous in character may later have a preponderance of small round cells in the fluid. In fluids due to mechanical causes the relative excess of endothelial cells is early and transitory. Later, the small mononuclears may predominate. The polymorphonuclear finely granular cells seem least subject to variation. Some cases with pleural effusion in which the small mononuclear leucocytes are clearly in predominance are at autopsy definitely non-tuberculous. In cases of malignant disease involving the pleura the fluid may at first resemble that due to mechanical causes, but in other cases the mulberry-like masses of proliferated endothelial cells (proliferative pleurisy) and the cells showing mitotic figures may aid in the diagnosis, while in some cases of sarcoma involving the pleura the numbers of spindle-shaped cells may suggest the cause. Lord, in 28 cases of pleurisy with effusion, found the small mononuclears from 90% to 100% in 24; 88% in 1; and from 70% to 75% in 3 cases. In the last three the epithelial leucocytes and the coarsely granular cells were prominent. If the fluid is complicated by pulmonary tuberculosis we may have a mixed infection and therefore a larger number of polymorphonuclear cells. In chronic passive congestion also there is a lymphocytosis; therefore a predominance of these cells does not always indicate tuberculosis, although it usually does. In cases of non-tuberculous pleurisy with clear serous effusion the polymorphonuclear neutrophiles may be in excess; in some, the small mononuclears predominate. The small mononuclears may predominate also in a mild pyogenic infection which is subsiding. In transudates the epithelial cells predominate, but if the fluid is of long standing, the small mononuclears. Secondary infection may confuse the picture. The fluid may also contain red blood-cells. Cholesterin, uric acid and sometimes sugar also are found. In its chemistry the fluid resembles blood plasma. The proteids are serum-albumin, serum-globulin, some nucleo-albumin and fibrinogen. The albumin will be over 4% in amount. The fluids may coagulate spontaneously.

Recently the predominance of eosinophile cells has attracted some attention. Bine[2] reported 2 cases of atypical pulmonary infection followed by pleural effusion. In 1 of these cases the blood contained 1% and the pleural effusion 51% of eosinophiles. Four years later, after the effusion had disappeared, the coarsely granular cells of the blood numbered 32%. In the second case these cells numbered 18% in the blood and from 30% to 40% in the pleural effusion. His conclusion is that an eosinophilia of the pleural effusion precedes that of the blood, reaches a higher point than in the blood and is the first to disappear. The etiology of the cases with pleural eosinophilia is so varied that this formula can have little importance in diagnosis. The condition has been found following trauma and in typhoid fever, pneumonia, tuberculosis, syphilis, polyarthritis, general sepsis, pulmonary gangrene, endothelioma, hemorrhagic infarct of the lung, cardiac insufficiency, influenza, etc. A pleural eosinophilia and basophilia not infrequently develop in cases of traumatic hemothorax. In some cases the eosinophilies number from 60% to 75% of the total cell count. They seem to be formed locally in the pleura and to be the result of a special transformation of leucocytic elements which can occur only in an aseptic medium. These cells later reach the blood and cause a tardy eosinophilia. It is believed that they are formed directly from the polynuclear finely granular leucocytes.

Bacteriology.—The pathogenic micro-organisms in pleural exudates may, if present, be demonstrated in cultures or even in smears made directly from the fluid as it is removed. In cases following influenza we have seen a perfectly clear serous fluid from which grew Micrococcus aureus. The demonstration of *Bacillus tuberculosis*, however, is exceedingly unsatisfactory because of the very small number of these organisms in the large volumes of the fluid. Jousett's method of *inoscopy* has been much used. At least 100 c.c. of the fluid are allowed to clot, the clot enclosed in sterile gauze is then washed in sterile water and transferred to a flask. Then are added from 10 to 30 c.c. of a digestive fluid consisting of pepsin 1 to 2 gms., pure glycerine and concentrated hydrochloric acid, of each 10 c.c., sodium fluoride 3 gms. and distilled water enough to make 1000 gms. This mass is digested in the thermostat at 38° C. until the clot has disappeared, which usually takes from 2 to 3 hours. This time may be shortened by occasionally shaking the mixture. The fluid is then decanted and smears are made from the sediment. Zebrowski recommends that 100 c.c. or more of the fluid be mixed with an equal amount of 1% sodium fluoride solution and kept in a cool place for 24 hours and then decanted. The sediment is then centrifugalized and smears made. Animal inoculation is, however, the only certain way of determining the presence of these bacilli. To be accurate, a large amount of the fluid must be injected into the peritoneal cavity of a guinea pig. According to some from 10 to 50 c.c. are injected each week until at least 300 have been introduced. The amount possible to inject will depend somewhat on its toxicity. The animal is then allowed to live for 3 months (unless it dies before then, in which case of course it is immediately autopsied, see page 27. At the end of this time the animal is killed and examined. Using this method Bacillus tuberculosis has been demonstrated in 85% of the cases of pleurisy with effusion (Le Damary said in 93%).

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[2] Am. J. of Med. Sc., 1918, vol. 155, p. 579.

Empyema.—In pneumococcus empyema which is primary or a sequela of lobar pneumonia the pus is creamy and sometimes so thick that none is obtained through a fine hypodermic needle. It is very rich in pus cells and usually contains many encapsulated diplococci. The pneumococcus found is usually type 1, 2 or 4. The empyema which develops during an attack of bronchopneumonia is an early rather than a late complication and so is often coexistent with the pneumonia, a matter of great moment in therapy since the evacuation of the fluid is a much more serious matter.

STREPTOCOCCUS HEMOLYTICUS EMPYEMA is the form of empyema which predominated in the army during the epidemic of 1918-19. It is a rare type in civil life. The fluid on aspiration has a light yellow color with a greenish tinge and at first is only slightly cloudy and slightly purulent. The cocci are numerous, large, round or slightly flattened and in chains of from 2 to 10. The cultures from the fluid on blood-agar plates show after 24 hours of incubation small, elevated, opaque colonies of a pearl-gray color, each surrounded by a sharply defined zone of hemolysis. Subcultures in blood broth give a diffuse and cloudy growth with a heavy sediment. The blood cells are rapidly destroyed therefore the broth becomes of a claret-red color. The smears of these cultures will show chains of streptococci.

In STREPTOCOCCUS VIRIDANS EMPYEMA the fluid is thin, grayish-red in color, turbid because of fine fibrin flakes and much later becomes truly purulent. These non-hemolyzing streptococci were found both in the sputum and in the pleural exudate of these patients.

In the INFLUENZA EMPYEMAS of 1917-20 the pneumococcus seemed to be the predominating organism and yet the pneumonia is very different from the ordinary lobar pneumonia since it was a very virulent necrosing bronchopneumonia. The influenza empyema developed late in the course of the pneumonia (in from the first [rarely] to the fourth week). These cases are frequently complicated by multiple metastatic abscesses in various parts of the body. The exudate is small in amount and when abundant enough to aspirate, often is too thick to flow through an ordinary trocar.

STREPTOCOCCUS EMPYEMAS sometimes complicate PNEUMOCOCCUS PNEUMONIAS. Of the 80 cases of Brooks and Cecil 9 showed this mixed infection, while in 1 case the emypema was due to Staphylococcus aureus. Streptococcus hemolyticus and Streptococcus viridans were found in these fluids, while the lobar pneumonia was due to the second, third and fourth type of the pneumococcus.

STERILE EMPYEMAS.—In about 20% of empyemas the fluid is sterile. In a majority of these cases it was purulent or semipurulent in character. Although the cultures are sterile, yet, in practically all cases disintegrated Gram-positive cocci can be seen in smears of the fluid. Most of these cases accompany a pneumococcus lobar pneumonia, the others, a streptococcus pneumonia.

In **hemorrhagic pleurisy** the exudate contains so much blood that it is grossly apparent in the fluid. Such fluids are met with in cases of malignant disease involving the pleural surfaces; in tuberculosis, in which case a blood vessel is supposed to be eroded by the tuberculous process; in various asthenic conditions including Bright's disease; in cancer in other parts of the body; in cirrhosis of the liver; and in rare cases of the malignant type of acute infectious fevers. In some cases of influenza a bloody pleurisy has appeared on from the fifth to eighth day of the influenza. The pleural effusion is almost pure blood. This character of the fluid seemed to have very little effect on the course of the disease. Bloody pleural effusions have been found also in healthy persons due to causes as yet undetermined.

Trauma of a blood-vessel by the needle used in aspiration is a common cause of the bloody effusion.

The pleural fluid of cases with carcinoma of the pleura is serous at first and possibly would remain so if not tapped, but later becomes blood-stained or hemorrhagic or stained by old blood and therefore of chocolate color. The amount of blood present would seem to depend in part on the degree of negative pressure used in the aspiration.

In cases of PRIMARY SARCOMA of the pleura there is usually an effusion present, hemorrhagic as a rule, or will be so at least after the chest has been once tapped. On microscopic examination of the sediment the discovery of spindle cells may suggest the diagnosis. This disease is uncommon.

The majority of malignant tumors of the pleura are secondary to those of the lung, mediastinum or chest wall, while others are metastases from any distant organ. These tumors are as a rule, small, separate nodules. The pleural fluid in such cases may be serous but is generally bloody.

In **actinomycetic infection** of the pleura this cavity usually contains pus in which can be seen grossly the sulphur granules (see page 45). Some of these are very minute in size, others even 2 mm. in diameter. They are white or yellow in color (hence the name "sulphur granule") and are irregular in shape and soft, but some are calcareous in texture.

Pericardial Fluid.—This fluid normally is of a lemon-yellow color, is slightly viscid and seems to contain more fibrin than do other physiological fluids. The solids it contains vary from 37.5 to 44.9 gms. per liter; the albumin, 22.8 to 24.7 gms.; soluble salts, from 8 to 9 gms., the insoluble salts, 0.15 gm. per liter, and extractives 2 gms. per liter.

The fluid of a recent tuberculous case had a specific gravity of 1.0204; solids 5.8%; total proteid 3.91% and globulin only 15.2% of this.

Synovial Membrane.—The synovial fluid is alkaline, thick, sticky, viscid, yellowish in color, and cloudy often from cell detritus; or it is clear.

It contains albumin, salts and a body which is physically like mucin, but which it cannot be, since no reducing body can be split off. Neither is it nucleo-albumin. Salkowski has given it the name "synovin."

The fluid from a recent case of rheumatism, and which clotted firmly, had a total proteid content of 4.3%; water-soluble extractives 1.07% (ash. 0.606%); alcohol-ether-soluble extractives 0.076% (ash, 0.046%) and fat fraction 0.35%.

Chylous and Chyliform Effusions.—Fluids which are milky in appearance because of a high fat content are called "chylous" fluids. These are evidently due to lesions of the thoracic duct or of its radials. Those whose milky appearance is due to large numbers of cells undergoing fatty degeneration are termed "chyliform". This distinction has, however, proven of little practical value, for the difference between these 2 kinds of fluids would seem to be of one degree rather than of kind. These effusions are rather rare. They have been found in patients of all ages and in 1 or several serous sacs. These fluids are milky-red (from the mixture of blood), yellow or green in appearance and odorless or with a slightly sweetish odor. They may in appearance resemble pus. If allowed to stand a definite cream may rise. The emulsion of the fat cannot be cleared by filtration or by centrifugalization, but will disappear if the fluid be shaken out with ether. Chemical analyses have shown the following:

Fat	0.06 to 3.71%
Albumin	3.36 to 7.37%
Total solids	5 to 10%
Organic matter	1%

Casein and fibrin have been found as well as cholestrin and lecithin. Glucose may be present in about the frequency and amounts that it is present in clear serous effusions. If more than 0.2% is present, however, this may indicate the thoracic duct as the source of the effusion. Microscopic examination shows the fat in fine emulsion and (in the chyliform cases especially) degenerating cells in great numbers.

Pseudochylous fluids are practically fat-free and owe their milky appearance to some unknown albuminous body. If allowed to stand these fluids remain homogeneously milky. In some cases the milkiness is due to albumin of the globulin group from which large amounts of lecithin can be extracted by hot alcohol. What fat is present resembles that of the so-called fatty degeneration of the epithelial cells. Quincke first showed that albumin in fine granules could give a fluid a milky appearance.

Some fluids become more milky on cooling. In some cases a perfectly clear fluid on the first tapping becomes progressively more milky on the subsequent tappings.

The specific gravity of these fluids varies from 1.010 to 1.014. In 1 case it was 1.061 and in another it was 1.081. In these cases much pus must have been present. Their reaction is alkaline and, strange to say, they have no odor. In the cases that we have examined this has been a marked feature. They are very resistant against decomposition and can remain in the laboratory for weeks without apparent change. The sediment is scanty, consisting of epithelial cells, all degenerated and full of fatty globules and globules which do not take the stains of fat.

Some of these cases of chylous effusion are due to filaria disease (see page 673) while in others the fat may best be explained by the fatty globules freed from the fatty degenerated epithelium cells. But the majority of cases are hard to explain. Our best case was one of tuberculosis of the peritoneum. Naunyn taught that amyloid degeneration of the vessels of the peritoneum was an important cause. In Tabora's case of peritonitis carcinomatosa the fluid contained 1.2% of fat and 0.864% of sugar. The opalescent fluids occur in a great variety of conditions and are often found at autopsy; in cachexias, heart cases, etc. The reason suggested for chylous fluids in cases of heart-failure is stasis in the thoracic duct. In other cases the stasis may be due to the pressure of tumors on the duct.

In one case ot markedly chylous ascites we studied the specific gravity of the fluid was 1.013 and the proteid 5.114 gms. per liter of which 73% was globulin. The fat-cholesterin-lecithin-fraction was 1.469%.

A chyliform ascitic fluid from a case of uremia had a specific gravity of 1.0055 and contained 1.2988% of solids.

OVARIAN CYSTS

Colloid as applied to the contents of ovarian cysts is not the name of one substance but of a group of fluids with certain physical properties, which is found in various cysts and organs. These fluids are gelatinous, the gelatinous substance insoluble in water and acetic acid but soluble in alkali. From some may be split off a reducing body but their composition varies much.

Pseudomucin (Metalbumin).—Pseudomucin occurs in many of the ovarian cyst contents which are very viscid and slimy. Alcohol gives a thready precipitate resembling wood-pulp, which can be wound around the rod. It is not precipitated by heat nor by acetic acid. The precipitate formed by alcohol is ground fine under alcohol and then freed from alcohol by means of ether, and dissolved in water. It is then reprecipitated with alcohol. A light white powder is obtained which is soluble in water to an opalescent mucoid solution, which is not well precipitated by acetic acid. When boiled with HCl an abundant reducing body is split off which reduces copper very easily.

Paramucin.—Paramucin is a substance present in certain ovarian cysts, also in the ascitic fluid providing the ovarian cyst has already ruptured into the abdomen. It is firm, glistening, with the consistency of gelatin, soluble in dilute mineral acid, shrinks in acidulated alcohol and in alcohol and ether, and can be reduced to a fine white powder. Its characteristics are: its insolubility in water, the fact that it swells in alkali dissolving in excess, that it is precipitated by acetic acid and is soluble in excess and, especially, that it will reduce copper salts without preliminary boiling with acid.

Serous Cysts (dilatation of the Graafian follicles) contain a perfectly clear serous fluid, watery, which foams easily, has an amber color and a specific gravity of from 1.005 to 1.022 (usually 1.005 to 1.014). It contains from 10 to 40 gms. of solids per liter and all the various constituents of serous fluids.

In 2 recent cases the specific gravity was 1.022 in 1 case and 1.016 in the other. They contained a great deal of albumin. Heat alone caused but a faint cloud, but 1 drop of acid made the fluid perfectly solid. Both serum globulin and serum albumin were present, but little or no euglobulin.

Proliferating Cysts from Pflüger's Tubules.—The contents of the cysts from Pflüger's tubules vary much. Some contain "colloid," which on boiling with acid gives a reducing body. From the colloid the fattycrystals (soluble on warming) may be watched as they crystallize out singly and in rosettes (see Fig. 149).

Another group of these cysts contain a viscid fluid, very stringy, which varies much in consistency. It is of a brownish or dark greenish-brown color.

The specific gravity of the contents of 2 cases was 1.025 and 1.0302; the solids were 9.7 and 9.3% and the alcohol precipitate, 6.9 and 8.5%.

Some, however, contain a thin watery fluid, of a bluish-white opalescent color which may, however, be yellowish, yellowish-brown, or greenish, according to the amount of blood present.

We give a few examples of the contents of such cysts which we have seen.

1. The fluid was quite opalescent; specific gravity, 1.0043; solids 2.837%. The alcohol precipitate was 1.98% of the fluid weight, resembled macerated filter paper, was not stringy, could be reduced to a fine white powder and was with difficulty soluble in water to an opalescent fluid. Watery extractives 0.524 (ash. 0.388)%, alcohol-ether soluble extractives 0.2056 (ash. 0.108)%; fat, cholesterol etc., 0.96%.
2. This fluid was reddish-yellowish in color and contained considerable sediment of small epithelial and some large epithelial cells with coarse refractile granules. It filtered clear. Its specific gravity was 1.0080, the solids 2.32% and the alcohol precipitate similar to the above.
3. This fluid had a bluish opalescent appearance and a specific gravity of 1.0073.

The contents of some of the multilocular cysts are thick, of a yellowish-red or brown color depending on the blood pigment, not specially viscid and contain a suspension of glistening masses of cholesterol crystals (see Fig. 150).

In a recent case the figures were: specific gravity 1.0259 and the alcohol precipitate, 10.56%; it contained no reducing body. Half saturation of the original filtered fluid with $(NH_4)_2SO_4$ gave a precipitate of 0.692% (globulin?): albumin (?) 0.604%, extractives, soluble in water, 1.46% (ash. 0.266%); alcohol-soluble extractives, 0.56% (ash. 0.44%). Microscopically there was a great amount of detritus in the sediment mixed with very large cells (epithelial) full of glistening granules, cholesterol crystals and fat needles.

In another similar case the specific gravity of the fluid was 1.0306.

The sediment contains much detritus, red blood-cells, leucocytes, large epithelial cells, single and in groups and filled with granules like fat, large masses of fatty granules, cholesterol crystals and colloid granules which are large, circular, strongly refractive bodies.

One DERMOID CYST contained much paramucin, also serum globulin and albumin. The water-content of the jelly of this case was 92.2%, its alcohol precipitate 3.3%, the water-soluble extractives, 0.4% (ash. 0.27%) and the alcohol-ether extractives 0.25% (ash. 0.16%).

Tubo-ovarian Cysts.—The contents of the tubo-ovarian cysts is a watery, thin, serous fluid which contains no pseudomucin.

Parovarian Cysts.—The parovarian cysts contain a thin, watery fluid which is colorless or has a very pale yellow color or is slightly opalescent. Its specific gravity varies from 1.002 to 1.009, the solids from 10 to 20 gms. per liter. It contains no pseudomucin. Albumin may be entirely absent or be present in traces only. These contain therefore only water and ex-tractives.

In a recent case, that of a patient aged 20 years, the cyst contained about 2 liters of very clear watery fluid with very slight opalescence. Its specific gravity was 1.0078, only the faintest precipitate with alcohol or ammonium sulphate could be obtained and the chlorides were 0.45% (as NaCl). Microscopically it contained very few epithelial cells, which were round and granular and with a round nucleus.

Intraligamentous Cysts.—The fluid of the intraligamentous cysts is yellow, yellowish-green, or brownish in color, contain little or no pseudomucin, its specific gravity varies from 1.032 to 1.036, its solids from 90 to 100 gms. per liter and the proteids it contains are those of the blood plasma.

HYDROCELE

The fluid of hydroceles is clear, dark yellow or greenish in color, the specific gravity varies from 1.014 to 1.026 and the solids average 60 gms. per liter. This fluid sometimes coagulates spontaneously. Leucocytes are always present in it and sometimes cholesterin crystals.

For illustration, in one case the specific gravity of the contents of the hydrocele was 1.0107; the solids, 6.329%; total albumin, 5.29% of which 45% was globulin; the water-soluble extractives were 0.7504% (ash. 0.462%); the alcohol-ether extractives, 0.452% (ash. 0.1726%); and the fat fraction 0.1864%.

SPERMATOCELE

The fluid of spermatocele cysts is colorless, watery and slightly milky. Its specific gravity is from 1.006 to 1.010. The solids average 13 gms. per liter, the proteids are slight in amount, while the sediment contains cells detritus, fat granules and spermatozoa.

The TOPHI of gout, so important in diagnosis, can be distinguished from small sebaceous cysts, small cartilaginous tumors, etc., only by the microscopic examination of their contents. A little of this mixed with water is found to be an amorphous paste containing many needles of sodium biurate (Fig. 151). The sebaceous cysts on the other hand contain amorphous sebaceous matter, containing many fatty and cholesterin crystals.

The masses of **urea crystals,** the "urea frost," which appear on the skin of the face just before death in rare cases of nephritis (there have been but 5 cases in this clinic) may be tested by the methods given on page 111. This is a most interesting phenomenon. The circulation in the skin is so poor when it occurs that it is very hard to believe that the immediate source of this urea can be the blood.

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